The Influence of Sitting Conditions on Soft Tissue Loads

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The Influence of Sitting Conditions on Soft Tissue Loads

Ph.D. Thesis

by

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Preface

This thesis has been submitted to the Faculty of Technology and Science at Aalborg University in partial fulfillment of the requirements for the Ph.D. degree in Biomechanical Engineering. The underlying work has been carried out at the Department of Mechanical and Manufacturing Engineering, Aalborg University during the period from June 2008 to March 2012.

The project was initiated by Hans Jørgen Brodersen to whom I would like to express my sincere thankfulness.

The project have been supervised by Associate Professor Mark de Zee and Professor John Rasmussen to whom I express my sincere gratitude for their competent professional guidance, support and friendship, which have helped me pursue the scientific goal.

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Christian Gammelgaard Olesen
Aalborg, March 2012.

Abstract

Pressure ulcer is a broad term for ulcers caused by a mechanical loading of soft tissue. Wheelchair users are in high risk of developing pressure ulcers under the buttocks. Knowledge about the aetiology behind the ulcers is limited and due to the nature of the problem, often it is too late to trace the cause of an ulcer. The aim with this work is to generate new knowledge about the aetiology behind sitting-acquired pressure ulcers under the buttocks.

The thesis comprises an overview of existing literature describing the current knowledge of the topic. The study initiates in a published literature review providing the basis for the following thesis. First the focus will be on how the seated posture affects the reaction forces between the chair and the person sitting on it. This part is investigated using a computer model of a seated human, developed in the simulation software AnyBody Modeling System. A validation study is presented of how well the chair reaction forces are estimated by the model compared to experimental data.

The seated AnyBody model calculates reaction forces for any given seated posture. The forces can be used as boundary conditions for a finite element model (FE-model) of a buttock. A study is presented where reaction forces estimated by the AnyBody model are applied to a FE model (CASIMIR/Automotive) that calculates the strain state in the soft tissue under the buttocks.

The strain state in the tissue is important due to strong scientific indications that it is directly related to the development of pressure ulcers. In order to investigate how the strain state relates to cell death, the last part of the thesis focuses on development of techniques for applying controlled deformations to cell cultures. The commercially available system Flexcell Tension 5000 was modified to apply uni-axial tension, and this was used to align growing muscle fibers on a flexible membrane. The purpose of the generated muscle tissue is to subsequently expose it to other mechanical stimuli. The final part of the work concerns application of different magnitudes of shear strain to the tissue in order to investigate its effect on cell death. Five scientific papers describe the above-mentioned areas and in further the understanding of pressure ulcer aetiology.

Abstrakt

Tryksår er en bred betegnelse for sår der opstår grundet en mekanisk belastning af blødt væv. Tryksår rammer blandt andet mange kørestolsbrugere under bagdelen. Viden omkring årsagen til at disse sår opstår er begrænset, da de ofte er svære at se før det er for sent til at spore den bagvedliggende årsag. Formålet med nærværende arbejde er at generere viden omkring etiologien bag tryksårs opståen under bagdelen i den siddende stilling. Afhandlingen indeholder et review af eksisterende litteratur der beskriver den nuværende viden på området. Der er taget udgangspunkt i litteraturgennemgangen i den efterfølgende afhandling, hvor første fokusområde er hvordan den siddende positur relaterer sig til reaktionskrafterne mellem stolen og personen der sidder på den. Denne del er undersøgt ved hjælp af en computermodel af et siddende menneske, udviklet i softwaren AnyBody Modeling System. Der bliver præsenteret et valideringsstudie, som viser validiteten af modellen ift. at beregne reaktionskræfter på en stol. Den siddende AnyBody model udregner reaktionskræfter, for enhver given siddestilling, disse kræfter kan bruges som randbetingelser for en finite element model (FE-model) af en bagdel. Der bliver præsenteret et studie hvor reaktionskræfterne udregnet af AnyBody modellen bliver påtrykt en CASIMIR/Automotive FE-model som udregner tøjningstilstanden af de forskellige væv under bagdelen. Tøjningstilstanden i vævet er vigtig, da det antages at denne er direkte relateret til udvikling af tryksår. For at undersøge hvordan tøjningstilstanden relaterer sig til celledød, er den sidste del af afhandlingen fokuseret på dette område. For at kunne udsætte levende celler for mekaniske belastninger blev der udviklet et setup til formålet. Der blev taget udgangspunkt i det kommercielle system Flexercell, som blev modificeret til at kunne påtrykke en-akset træk, dette blev brugt til at få muskelceller til rette sig i samme retning på en fleksibel membran, som efterfølgende kunne bruges til at påtrykke andre mekaniske stimuli. Den sidste del består i at påtrykke forskellige grader af forskydningstøjninger, for at undersøge hvilken effekt forskydningstøjninger har på celledød. Fem videnskabelige artikler beskriver de ovennævnte områder, og giver et billede af, hvordan etiologien bag tryksår kan undersøges videre.

Publications

The entire work during the Ph.D. period has spanned a broad area ranging from tissue engineering in cooperation with Aalborg University's Stem Cell Lab to computational investigations applying the developed methods to unrelated areas like ankle biomechanics. The chapters included in the thesis report only on the part of the work that is central to the problem field of pressure ulcers, while the publication list contains references to the entire scientific production during the Ph.D. study at the Department of Mechanical and Manufacturing Engineering, Aalborg University.

Publications in refereed journals

- Olesen C.G, de Zee M, Rasmussen J. (2010): Missing Links in Pressure Ulcer Research - An Interdisciplinary Overview. *Journal of Applied Physiology.* **108**(6), 1458-1464
- Nielsen R.G, Rathleff M.S, Moelgaard C.M, Simonsen O, Kaalund S, Olesen C.G, Christensen F.B, Kersting U.G. (2010): Video Based Analysis of Dynamic Midfoot Function and its Relationship with Foot Posture Index Scores. *Gait & Posture*, **31**(1), pp. 126–130.
- Rathleff M.S, Nielsen R.G, Simonsen O, Olesen C.G, Kersting U.G. (2010): Perspectives for Clinical Measures of Dynamic Foot Function - Reference Data and Methodological Considerations. *Gait & Posture*, **31**(2), pp. 191–196.
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- Olesen C.G, de Zee M, Rasmussen J. (2011): Elliptical posts allow for detailed control of Non-Equibiaxial straining of Cell Cultures *Journal of Applied Physiology.* **Under review**
- Pennisi C.P, Olesen C.G, de Zee M, Rasmussen J, Zachar V. (2011): Uniaxial cyclic strain drives assembly and differentiation of skeletal myocytes. *Tissue Engineering. Part A. Tissue Engineering*, **17**(19-20), pp. 2543-2550
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- Olesen C.G, de Zee M, Rasmussen J. (2009): Experimental validation of a computational human seating model. *Proceedings, XXIInd Congress of of the International Society of Biomechanics*, 5-9 July 2009, Cape Town, South Africa.
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- Olesen C.G, Busscher L, de Zee M, Rasmussen J. (2010): The significance of passive stiffness to a computational musculo-skeletal spine model of a seated human. *Abstract from Computer Methods in Biomechanics and Biomedical Engineering*, 23-27 Feb. 2010, Valencia, Spain.
- Olesen C.G, de Zee M, Rasmussen J. (2010): Using a computer model to bridge published experimental seated posture results. *Abstract from 13th Annual European Pressure Ulcer Advisory Panel Meeting*, 1-4 Sept. 2010, Birmingham, Great Britain.
- Olesen C.G, de Zee M, Rasmussen J. (2010): Why is the Etiology of Pressure Ulcers Still Unknown? *Abstract from 26th International Seating Symposium*, 10-13 Mar., Vancouver, Canada.
- Olesen C.G, Lund M.E, Sloth S, Heinen F, Nedergaard N.J, de Zee M (2011): Development of a musculoskeletal model for coffee-grinder design purposes. *Program & Abstracts, 3rd Annual Meeting of the Danish Society of Biomechanics* 14 Oct. 2011, Univ. Southern Denmark, Odense, Denmark.

Contents

1

Introduction

Pressure ulcer (PU) is a broad term used for describing ulceration of tissue caused by mechanical loading (Romanelli et al., 2005; Schroeder, 2005). A PU affects hospitalized patients' or wheelchair users' quality-of-life significantly (Gorecki et al., 2009). The prevalence and incidence rates of patients affected by PU are difficult to describe due to lacking definition of the problem, however the latest prevalence study conducted at ten Danish hospitals in 2005 found that approximately 33% of all the hospitalized patients at the time had a pressure ulcer (Bermark, 2009). With respect to the wheelchair users, statistics show that nearly all wheelchair users will, at some stage in their lives, experience a pressure ulcer (Chen et al., 1999; Salzberg et al., 1996). Besides the personal traumas experienced by the patients, the socio-economic impact is also significant. Bennett and colleagues (2004) estimate that the cost of treating one pressure ulcer in the UK National Health Service (NHS) ranges between £1,064 and £10,551 (€1,250 – €12,398) and that the total costs for this treatment comprise 4% of the total NHS budget, i.e. in the billion Euro range for the UK alone (Bennett et al., 2004).

1.1 Types of Pressure Ulcers

Pressure Ulcers can be divided into different categories in order to monitor and document the healing process. The recently published recommendations of the European Pressure Ulcer Advisory Panel (EPUAP) and National Pressure Ulcer Advisory Panel (NPUAP) divide the ulcers into four categories ranging from redness of a localized area to full thickness tissue loss and exposed bone (EPUAP, 2009). PUs can be localized at most places on the body. However some places are more prone to PU, such as the heels, sacrum and iscial tuberosities. The position of the wound varies from bedridden hospitalized patients to wheelchair users.. This thesis will focus on PUs typical for wheelchair users, especially the so-called deep tissue injuries which are considered especially harmful because muscle and subcutaneous tissue may suffer substantial necrosis, while only minor signs of tissue breakdown are visible on the skin (Agam and Gefen, 2007). Therefore, Sitting Acquired Deep Tissue Injuries (SADTI) will be the main focus throughout this thesis.

1.2 Risk Factors

Wheelchair users are subject to a number of different risk factors. Besides the mechanical loading of the tissue, many other factors determine the influence of compression and shear forces on tissue: Elevated body temperature, moisture from perspiration, malnutrition, dehydration, incontinence, Alzheimer etc. These risk factors are taken into consideration in several risk assessment tools, but their interplay is not well understood. Different scoring systems for assessing the combined effect of risk factors are available (Braden, Norton, Waterlow) and used to interventions such as pressure-distributing cushions and supporting aids (Beeckman et al., 2007; Refshauge and Fitzpatrick, 1995; Refshauge et al., 1995). Most of the risk factors listed above, such as body temperature, moisture, malnutrition, dehydration etc. will not be investigated in this thesis. The risk factors in focus will be the mechanical factors such as reaction forces, deformations etc.

1.3 Prevention of Pressure Ulcers

In practice occupational and physiotherapists are responsible for fitting wheelchairs to the users, and also for choosing the supporting aids and cushions. Choices are often based on clinical experience and "know-how". Also educating the wheelchair user about basic principles of pressure ulcer prevention is used at many rehabilitation clinics with good results. This should give the wheelchair user an idea of how to minimize the risk of developing a pressure ulcer during normal daily activities. Research and "know-how" in the PU area have improved over the last decades, but the number of pressure ulcer incidences is still very high and the effect of prevention varies much between patients, therapists, institutions and geographical locations. In order to consistently prevent SADTI, it is important to fully understand the underlying mechanisms, i.e. convert know-how into know-why.

1.4 General Problem Formulation

The problem in focus in this thesis will be to take a step towards a better understanding of how the seated posture affects the tissue deformation and consequently tissue necrosis. Chapter 2 contains a review paper gathering a century of research results and summarizing them to three focal points that should be investigated further to gain a better understanding of the aetiology of SADTI. The nature of the problem includes large statistical variations and purely empirical data struggle to provide statistical significance due to experimental noise. The approaches chosen will therefore combine different computational models for investigating the general problem and providing insight into the basic mechanisms, and experiments for verifying the computational investigations.

The studies reported in subsequent chapters include focus on how different seated

postures change the reaction forces deforming the tissue under the buttocks during sitting. The clinical relevance of this work is that wheelchair adjustment remains one of the frequent interventions used by therapists to prevent and promote healing of pressure ulcers. The reaction forces between the buttocks and the seat lead to strains in the deep tissues where necrosis is initiated to form a SADTI. However, material parameters as well as the geometry of internal interfaces between different tissue types are complex and this is also the case for the resulting strain state. To gain insight into the connection between external loads and internal strain states, a finite element model of the buttock is acquired, further developed and applied.

This result would ideally be linked with experimental results on the relationship between tissue strain state and cell necrosis, thus forming a full causality between sitting environment and ulceration risk.

Thesis Outline

- Review of literature
- Project plan
- Postural Effects
- Buttocks Tissue Deformation
- Cell Death Criterion
- Discussion and Summary of Papers

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Paper 1: Missing Links in Pressure Ulcer Research - An Interdisciplinary Overview

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Missing links in pressure ulcer research—An interdisciplinary overview

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Olesen CG, de Zee M, Rasmussen J. Missing links in pressure ulcer research—An interdisciplinary overview. *J Appl Physiol* 108: 1458–1464, 2010. First published March 18, 2010; doi:10.1152/japplphysiol.01006.2009.—This paper surveys the literature on the etiology of sitting-acquired deep tissue pressure ulcers from three different viewpoints. The first viewpoint is identification of risk factors related to seated posture. The second viewpoint focuses on the external factors that can cause necrosis to human cells, such as ischemia and compression. The third viewpoint focuses on computational models of the human buttocks to calculate where stress concentrations occur. Each viewpoint contributes to the understanding of pressure ulcer etiology, but in combination they cover the multiple scales from cell to organism, and the combined insight can provide important information toward a full understanding of the phenomenon. It is concluded that the following three questions must be answered by future research. *1*) Does compressive stress alone explain cell death, or is it necessary to consider the full three-dimensional strain tensor in the tissues? 2) How does the change in posture-induced load applied on the human buttocks change the stress distribution in the deep muscle tissue? 3) Is it possible to optimize the seated posture in a computational model to reduce the deeper tissue loads?

wheelchair; biomechanics; deep tissue injury; spinal cord injury; pressure ulcer

PRESSURE ULCERS on the buttocks affect nearly all wheelchair users (47) and are a major cost factor in the healthcare system (35a). Pressure ulcers are difficult to prevent and challenging to treat. Classical treatment involves extended periods of bedrest (12), which is believed to cause further deterioration of the patient's general condition (7) and may lead to formation of ulcers in new places and even to sepsis or other potentially lethal complications (18).

"Pressure ulcers" is a general term covering a number of different tissue injuries, from superficial heel sores to deep pressure sores under the buttocks. A general introduction to the field of pressure ulcer research has been combined in a comprehensive book by Bader et al. (4). In this paper the focus will be on the understanding of how sitting-acquired deep tissue injury (SADTI) develops. The paper will not concern prevention or treatment of pressure ulcers in general. SADTI is rooted in the deep tissue under the buttocks, frequently in the interface between muscle and bone, and it is the type of ulcer that affects wheelchair users the most, despite active prevention and treatment. Wheelchair users with spinal cord injury are often affected by ulcers that originate in the buttock area (16). However, in general, it is difficult to assess whether a specific ulcer originates from the deep tissue or not.

There is an agreement that SADTI are due to necrosis in the soft tissues under the buttocks and that the initial cause is mechanical loading. However, soft tissues in healthy individuals are subjected to many types of mechanical loading during activities of daily living that do not lead to formation of ulcers, for example, sitting in office chairs, riding a bike, etc. The detailed mechanism behind SADTI remains unknown, and, as Agam and Gefen (3) point out, this is a major obstacle for the prevention and treatment of the condition.

For the purpose of systemization, we shall group the literature in the field of SADTI etiology into the following categories depending on the focus of the investigations: *1*) a biological approach that seeks to understand cell necrosis under different conditions; *2*) finite element models of tissue deformations and stresses; and *3*) experimental investigations of the influence of posture.

A large body of literature on each of the above topics is available, but a full explanation of the etiology behind the formation of pressure ulcers has not been found. In combination, the three categories cover the multiple scales from cell to organism, and the combined insight can provide important information toward a full understanding of the phenomenon. Thus, to make progress, there is a need for an overview that encompasses all of the contributing fields.

The objective of this paper is to review the literature that contributes to an understanding of the formation of pressure ulcers and propose avenues of future research that can improve our understanding of the phenomenon.

THE BIOLOGICAL APPROACH

While there is little doubt that pressure ulcers are related to the mechanical loading of soft tissue, several hypotheses have been developed pertaining to the link between the mechanical

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loading and the tissue necrosis. The two major hypotheses deal with ischemia and tissue deformation.

Ischemia Hypothesis

Ischemia is a state caused by lack of blood supply to any given tissue. The hypothesis proposes that a mechanical loading of the tissue impinches the arterial blood vessels, thereby causing local ischemia. Since cells depend on oxygen, heat, and nutrients transported by the blood, they will become hypoxic and subsequently necrotic. Thus the relevant questions are how much time it takes for ischemia to cause necrosis in the affected cells and how much necrotic tissue it takes to initiate an ulcer?

Already in 1922, Brooks (10) showed on dogs that ligating the primary artery to a muscle does not cause inflammation even when the state is maintained for several days. However, the affected muscles showed very fast signs of fatigue when used, indicating that the ligation worked. Subsequently in the same study, the vein was ligated, which in theory causes the same reduction of blood flow through the muscle as ligation of the artery, but was observed to cause significant necrosis. The authors hypothesized that the pressure would increase to somewhere between the diastolic and systolic pressure and cause ruptures of the capillaries and thereby edema followed by inflammation and necrosis.

Early specific investigations of pressure ulcers were performed by Michael Kosiak et al. (35), starting with an early version of a pressure measurement apparatus in 1958. The pressure distribution measurement tool is widely used in various clinical setups today, but it is important to understand that it has limitations that were not considered initially. Kosiak's work refers to Brooks' study (10) and was based on the hypothesis that tissue necrosis is caused by ischemia. This was investigated with animal models to which pressure was applied and tissue necrosis observed. The amplitude of pressure and time duration were varied for different animals, and a relationship between time and pressure was established (33, 34). Kosiak's experiments verified that pressure causes ulcers but did not reveal whether the effect is related to ischemia caused by the pressure. Ischemia remains a popular explanation of pressure ulcer etiology to this day.

Experimental approaches using animal models have also been employed by several more recent researchers (15, 33, 36, 44, 52). In these studies pressure was applied to the skin and muscles of rats and pigs. Daniel et al. (15) observed that, when studying pressure ulcers by means of an animal model, it is important to choose an animal, such as a pig, whose skin and subcutaneous tissues have similar mechanical and physiological properties to the human (13). Pressure ulcers were induced into the tissue covering the greater trochanter of the pigs. The principal finding was that the muscle tissue breaks down before the skin.

A study conducted by Zhang and Roberts (60) showed that a surface shear force decreases the blood flow more than a normal force of the same magnitude. Earlier, Bennett et al. (5, 6) had constructed a sensor that could measure pressure, shear force, and blood flow on the skin. The sensor was first tested on the soft tissue in a hand (5) and then later used for experiments underneath the buttocks of normal individuals, geriatric individuals, and paraplegics (6). It was found that the shear force in seated geriatric and paraplegic patients was roughly three times higher than typical values for healthy individuals. The median rates of pulsatile skin blood flow for geriatric and paraplegic patients were only one-third of the normal values. The seated posture of the test subjects was not registered.

Goossens et al. (29) used a transcutaneous oxygen/CO_2 monitoring system to investigate the influence of combined pressure and shear loading on ischemia. The sensor was indented into the skin either by pure normal pressure or by a combination of normal pressure and shear stress of 3.1 kPa, and the skin oxygen pressure was measured simultaneously. With pure normal loading, a mean applied pressure of 11.6 kPa was required to obtain a skin oxygen pressure of $<$1.3 kPa, which was presumed to be the ischemic limit. When a shear stress of 3.1 kPa was included in addition to the normal pressure, a normal pressure of only 8.7 kPa was necessary to reduce the oxygen pressure to 1.3 kPa, thus indicating that shear has an influence on ischemia. However, the combined normal force, shear force, and deformation of the skin create a stress state in the underlying tissues. This stress state is highly localized and complex and does not necessarily separate into shear and normal components according to the applied loads, and this makes interpretation of the result difficult.

The results of Zhang and Roberts (60) and Goossens et al. (29) show that applied pressure and shear forces decrease the blood flow in the skin and some time after the pressure is relieved the blood flow will return to its previous level. It is possible that this recovery time may prolong hypoxia enough to cause necrosis. This was studied by Meijer et al. (43) in an investigation of 109 elderly individuals, and it was concluded that the blood-flow recovery time is a direct measure of susceptibility to pressure ulcers.

Other research groups hypothesize that it is the reperfusion of the tissue after an ischemic event that causes cell death. Several research groups have conducted experiments and shown that reperfusion harms the tissue more than the actual ischemia (46, 56). The reperfusion is positively correlated to the duration of the ischemia (54).

A recent study by Deitrick et al. (17) worked from the hypothesis that spinal cord injured (SCI) patients get insufficient exercise due to the paralyzed muscle mass in the lower extremities and therefore are prone to poor circulation in the legs, which represents an increased arteriosclerotic risk factor. The hypothesis was examined by measurement of the blood flow to the legs in SCI patients and an able-bodied control group. It was shown that SCI patients in general have less blood flow in the legs, which might explain the poor healing rate observed in many SCI patients.

In conclusion, the ischemia research is characterized by an experimental approach in which the correlation between external factors, such as applied skin load, and measurable conditions, such as blood flow, is investigated. The findings are that pressure and shear force can decrease the blood flow in the skin of humans, but for obvious reasons induction of necrosis is never the intended outcome of these experiments. The animal studies show that necrosis starts earlier in the muscles than the skin. Experimental studies are the best source of information about the correlation between external loads and measurable quantities such as blood flow in humans and induction of necrosis in animal models. However, it is difficult to investi-

gate the correlation between the external loading and the cell death caused by ischemia purely by experimental means.

Deformation

A number of scientists have worked from the hypothesis that deformation of cells in its own right can cause individual cell death leading to necrosis. In one of the earlier studies, Husain (32) conducted experiments with rat muscles subjected to two fundamentally different types of mechanical loading: hydrostatic pressure and a mechanical point loading. The latter generates a complex combination of tension, compression, and shear stresses in the tissues. The results showed large differences in the amount of pressure the cells could withstand, depending on the loading conditions. The cells were in general very robust against hydrostatic pressure, whereas the point load caused necrosis.

In a study by Breuls et al. (8), pressure was applied to engineered muscle tissue by means of a round indenter causing a homogenous stress field under its surface and local stress concentrations close to the edge of the indenter. Necrosis was observed especially near the edge and the observation was made that this is due to the stress concentration, but the experiment did not reveal the influence of each of the six components of the stress tensor. The possible influence of shear stresses was therefore not addressed.

Systematic studies by Gawlitta et al. (20, 21) have investigated how much hypoxia and compression, respectively, contribute to muscle tissue necrosis. It was found that hypoxia does not lead to tissue damage within the first 22 h, whereas a compression of 30–50% strain leads to cell death within a few hours. Furthermore, hypoxia did not add additional effect to compression within this time. Stekelenburg et al. (52) conducted indenter experiments on rats while using MRI scans and histology at several time points to assess the degree of necrosis. They concluded that the deformation was what caused necrosis in rat muscles, where the perfusion damage was reversible.

Although the study of deformation does not support the ischemic hypothesis, time might still play a significant role. Gefen et al. (24) showed that there is a relationship between compressive strain and time, meaning that time does play a role in the deformation hypothesis and therefore should be considered in future models using the finite element method. This hypothesis was also supported by Linder-Ganz et al. (36) who showed a relationship between time and pressure in rat models. Studies have also been conducted on an even smaller scale. Peeters et al. (45) developed a setup for investigation of the deformation of a single cell. When applying a unilateral compression to the cell, a constant volume indicating an incompressible state of deformation was observed. It was also observed that this leads to an increased surface area. In a recent study, Slomka et al. (51) developed a finite element model (FEM) model of a human cell subjected to pressure and concluded that the tensional stress in the membrane was high when the cell was compressed. This led to the hypothesis that tension in the plasma membrane increased the influx of Ca^{+}, leading to cell death.

In summary, the research within this area finds that deformation causes cell necrosis much faster than hypoxia. It is also revealed that there is a time/strain relationship, meaning that time does indeed play a role. As early as 1953 Husain (32) showed the importance of the loading conditions with regards to muscle damage, but only one attempt by Linder-Ganz and Gefen (37) linked the findings with the stress states induced by the external loading of the tissue.

FINITE ELEMENT MODELS

Modeling of soft tissue deformation underneath the buttocks has usually been done by means of the FEM, and several models have been constructed using various approaches. One of the earlier models is by Todd and Thacker (55). The purpose of this model was wheelchair seat cushion design. The geometry of the model was based on MRI scans of two men. The material properties of the different tissues were identified by matching loaded and unloaded images with model predictions. It was concluded that FEM can be used to optimize the seat cushion, but the study did not take the influence of the external loading into account. The model was loaded only by a vertical compression, while the shear forces that are considered major risk factors by other authors (28, 29, 60) were neglected.

A general model explaining the pressure ulcer problem in the pelvis, ankle, head, and shoulder area was developed by Linder-Ganz et al. (28, 38). This model was based on the "Visible Human" database (19, 58a), and the mechanical properties of the different tissues were estimated from experiments with rats. The Visible Human specimen was frozen in a supine posture and the model therefore entails assumptions about the soft tissue deformation from supine to seated posture.

External loads were calculated from free body diagrams and applied to the model. These calculations take gravity into account but neglect the contribution of muscle forces. It was found that a backward inclination of the backrest decreases the internal stress in the deep tissue under the buttocks, leading to the conclusion that internal stresses in the deeper tissues are sensitive to posture. In a subsequent study, Gefen et al. (23) investigated the transient mechanical properties of rat muscle tissue and applied their findings to the model of Linder-Ganz et al. (38). It was found that the muscle tissue becomes stiffer over time due to compression, causing 30–40% increased compressive stress in the deep muscle tissue after 4 h.

Another study by Gefen et al. (22) also used the muscle stiffening found in Gefen et al. (23). A computational FEM was used to show a relationship between inclination of the backrest and the time of tissue injury onset. The load transferred through the ischial tuberosities to the gluteus muscle for different backrest inclinations was calculated by the formula:

$$L = \frac{\phi \cdot BM \cdot g}{2} \sin(180° - \alpha)$$

where L is the load transferred through the bones, BM is the body mass, φ is the weight fraction of the upper body, and α is the backrest angle. However, depending on friction coefficients of the seat and backrest, a shear force would also have been present but not included in the calculation.

Another FEM of the human buttocks was constructed by Linder-Ganz et al. (39). This model was constructed by means of a double MRI device enabling scanning of a seated posture, i.e., with the soft tissues deformed to their final positions. One scan was performed with the subject floating on a rubber tube with no load on the buttocks and another scan had the subject seated on a chair. The two scans were compared and the

stresses were calculated from the observed deformations in six healthy individuals. The seated position attained by the subjects during the scans was unreported and it is not clear from the paper whether a backrest was included. The setup was also used by Linder-Ganz et al. (40), where it was concluded that paraplegic patients suffer from higher than normal stress states within the gluteus maximus muscle. A similar model was developed by Then et al. (53). It is a general buttock model constructed from an MRI scan in an unloaded situation. The material properties were estimated from MRI scans performed while an indenter was pressed into the soft tissue at various locations on the buttocks. The deformation was then used in a reverse engineering approach to determine the material properties leading to the observed deformation. This model can also be used to evaluate cushion design with respect to internal stresses. The advantage of this approach is that the anatomy and material properties derive from the same subject.

Buttock models based on FEM have also been used in other research areas, for example, in design of comfortable automotive seats, and several models have been developed for this purpose. One model is the so called MADYMO seated model, which is a three-dimensional FEM originating from Verver et al. (58). The model was developed with the automotive industry in mind, and vibration was the main focus. The model does not distinguish between the different types of soft tissue, as the internal stress state of the tissue is not considered the main focus in automotive comfort analysis. A somewhat similar approach was used by Siefert et al. (50) and has resulted in the commercially available model CASIMIR, which does distinguish between the different soft tissues, although the focus of the model is vibration and not tissue stress.

The finite element method offers an opportunity to resolve the internal stress state in the tissues that is not available from purely experimental approaches. However, the model construction is complex and does not easily lend itself to individualization reflecting the anatomical changes an SCI patient undergoes, such as bone demineralization and muscle atrophy (25). Linder-Ganz et al. (40) created individualized models of muscle atrophy and found that paraplegics suffer from higher stresses within the gluteus maximus. However, to obtain seated posture tissue data, the model individualization required input data from a double-donut MRI scan.

The different tissues, their respective material properties, and the complexity of the geometry make the construction of an FEM a tremendous effort. Furthermore the models involve large three-dimensional meshes, large deformations, nonlinear material properties, and contact conditions and consequently long computation times. Therefore these models are not practical in a clinical setting.

The knowledge that large-scale FEMs can contribute is important for the understanding of the etiology of pressure ulcers. Although the work load of constructing a detailed FEM is tremendous, several models have been developed and complex material properties have been identified for use in the models.

For clinical purposes, Linder-Ganz et al. (37, 41) used a parametric FEM simplified into a single slice of tissue based on an MRI scan with pressure mapping as boundary condition and validated it against a 3-D FEM and measurements on a physical model of the buttocks. Another tool based on Hertz contact pressure was developed by Agam and Gefen (1) taking advantage of the closed form solution to Hertz' problem. The tool can take individualized parameters as input, such as thickness of muscle layer, radii of ischial tuberosities, and stiffness modulus. This model too is validated against physical model measurements and finite element computations.

Some researchers have combined a cell death criterion with the stress state of an FEM. The criterion related cell death with compressive stress (36), which is the intuitive stress type, given the external loading conditions. It was demonstrated that the more atrophic muscles had higher necrosis compared with nonatrophic muscles (37).

Wagnac et al. (59) built an FEM that allows morphing of the pelvis geometry from bony landmarks on tomography images and morphing of the external shape of the soft tissue to markers placed on the skin. However, compared with the preceding models, the multiple material properties of the soft tissues have been lumped into a single soft tissue material. This setup makes it possible to use the model in the daily practice but it is unclear which inaccuracy is introduced by the simplifications.

The internal stresses computed by FEMs depend entirely on the applied loads. Several experimental investigations indicate that the posture influences the tissue loads significantly and clinical experience has caused therapists to consider the shear force as one of the most important risk factors (28, 29, 60). Wheelchair patients often require the support of the backrest to remain stable in the seat. Leaning against a backrest will, according Newton's third law, cause seat shear forces in the opposite direction. Despite this, only the model by Linder-Ganz et al. (38) attempts to take the dependency between seated posture and loads on the model into account. However, even in this model, the loads are only the passive forces originating from gravity, while the contribution from the muscle forces necessary to maintain the seated posture is neglected. Thus the FEM approaches do not completely bridge the gap from external seating conditions to ulcer formation.

Posture

A significant body of research has focused on the seated posture from the hypothesis that posture influences the formation of pressure ulcers (9, 14, 26, 27, 31, 42, 57).

The work typically assumes some measure of the experimental output as an indicator of risk, while changing seated posture. Gilsdorf et al. (26, 27) looked at the normal and shear forces measured by a seat-mounted force plate. The forces were recorded while changing seat and backrest angle, and the shear force was hypothesized to be a key risk factor.

One year later Hobson et al. (31) conducted a similar experiment, but instead of measuring reaction force an early version of a pressure measurement tool was used to assess the pressure and pressure gradient. The shear force was also measured. Subsequently, the posture was changed by means of the backrest and seat, and mean pressure, peak pressure, peak pressure gradient, and shear force were assumed as risk factors. The findings were that paraplegic patients have higher peak pressures and peak pressure gradients than able-bodied subjects but it is difficult to assess these measures in terms of ulcer risk.

Also Shields et al. (48) attempted to relate the posture to the seat pressure distribution by developing and employing one of

the first pressure distribution systems to show that the peak pressure underneath the buttocks decreases when adding a lumbar support.

In a more recent experiment, Maurer and Sprigle (42) investigated whether a smaller angle between the seat and backrest, i.e., an angle less than 90 degrees, increases the perpendicular interface pressure. This seat adjustment is known as "squeezing" the chair and is used in clinical practice to increase stability for SCI patients. No difference in the pressure distribution was found and it was concluded that squeezing is not a problem. However, the pressure gradient was not considered and neither was the fact that the changed seat angle is no longer perpendicular to the gravity field and therefore will not comprise the tangential component of the force.

Another study by Brienza et al. (9) showed that there is a relationship between peak pressure and the incidence of pressure ulcer development in elderly wheelchair users. The same results were concluded by Conine et. al. (14).

In a recent publication dealing with the relationship between posture and sitting-acquired pressure ulcers, Geffen et al. (57) report on the construction of a chair with the ability to control the angles between the lumbar spine, the pelvis, and the hip. In an experiment those three angles were varied while measuring interface pressures and reaction/shear loads. A good correlation between some parameters is demonstrated, providing valuable guidelines for clinical practice and for validation of computer models of the phenomenon.

There have been other experiments working with posture and how different postures affect the loads between a chair and a subject. For instance, Bush and Hubbard (11) experimented with measurement of support forces in different seated positions. The study was related to car seats, but it adds an understanding of how change in posture affects the support forces. The study concludes that shifts in posture affect the force distribution on the seat significantly.

Shirado et al. (49) studied the movement of center of pressure (COP) during long-time sitting in paraplegics and able-bodied without backrest support and found that COP for paraplegics moves significantly more than for able-bodied individuals due to the reduced balance and stability of many SCI patients. Practically attainable postures are therefore limited by the individual's ability to retain stability and cannot be selected from pressure ulcer considerations alone.

Much research in the postural tradition seems based on the premise that posture influences support forces and forces cause ulcers. Thus the field focuses on experimental investigation of the correlation between posture and forces. Many valuable contributions have been made in this area, especially concerning the relationships between forces and postures. Many contributions have also been within pressure mapping and the relations to postures. All in all very valuable knowledge for therapist working with SCI wheelchair users. This useful knowlegde, however, does not lead to an explanation of how the support forces may influence ulcer formation except that more force likely leads to more ulcers.

DISCUSSION AND CONCLUSIONS

The reviewed literature reveals valuable contributions from different approaches, each contributing an aspect to the understanding of pressure ulcer etiology. This overview has focused on research questions related to risk factors for wheelchair users, FE buttock models, ischemic experiments, and cell culture experiments. The findings reveal that high interface pressure and shear forces are risk factors for wheelchair users. The FEMs have shown that interface pressure leads to strain and stress concentrations in the interface between bone and muscles under the buttocks. The experiments investigating ischemia have shown that pressure impinches arteries, which does not quickly lead to necrosis, while impinchment of the vein does. The tissue experiments have shown that deformation rather than hypoxia kills the cells.

Despite these valuable contributions no single approach can explain the phenomenon from overall body posture to cell-level injury. The field still faces challenges to uncover the interplay between these factors, i.e., the road from gravity via posture and chair support to stress and deformation state in the tissues ending with possible necrosis.

It is a remarkable finding (20) that cells die from mechanical loading directly and not due to the ischemia it causes. However, doubt remains concerning the severity of different types of deformation the tissue may be subjected to, and additional investigation of how the individual components and combinations of components in the stress and strain tensors may influence the tissue is required. There is a significant need for development of a multi-dimensional cell death criterion akin to failure criteria from solid mechanics.

With a cell death criterion in place, the two missing links between sitting conditions and tissue necrosis seem to be from posture to net pressure and from net pressure to stress state. Both of these are difficult to obtain from experimental models alone, although good studies have been carried out, showing relationships between pressure measurements and pressure ulcer incidents. Pressure mat experiments do not provide information about shear forces, and force platforms are difficult to integrate fully into realistic seats. Furthermore, measurements of external pressures or forces do not reveal much information about the internal deformation state in the tissue.

Computational models appear to be able to bridge some of these gaps. A musculoskeletal model simulating in vivo muscle forces, the chair, and the boundary conditions might capture the complexity of force exchange between the body and the chair for different seating conditions. The output of the musculoskeletal model can be validated against the data from postural experiments.

The reviewed FEMs were loaded only by forces perpendicular to the seat surface; no shear forces were applied to the models. This seems to contradict the hypotheses and findings of the clinical and posture-related research concluding that shear force is a key risk factor. Thus a finite element model of the soft tissues working from correctly estimated interface forces including shear from a validated musculoskeletal model can compute the resulting localized tissue stresses for input into the cell death criterion, leading to a prediction of necrosis under different sitting conditions.

In conclusion, pressure ulcer research is a highly interdisciplinary area requiring a combination of approaches and a combination of results by which the field can progress and a full understanding of this important clinical problem may be obtained.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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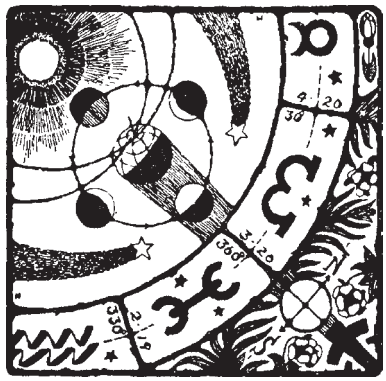

3

Project plan

The review paper of the previous chapter describes state-of-the-art in the field as of 2010. It emphasizes recent results indicating that deformation of the cells is the main initiator of necrosis. Three missing links were pointed out, and they will be the focus of the subsequent papers. The missing links were the following:

1. The first missing link is rooted in the therapeutic approach to PU management and is a lack of understanding of how postural changes affect the support forces acting between the chair and the person sitting on it. This problem will be investigated using a computational musculo-skeletal model of the human body sitting on a chair. The advantage of this approach is that the knowledge gained will be general and not disturbed by experimental noise. The disadvantage is that the model is only a limited representation of the "real world". Therefore a validation study of the reaction forces estimated by the model is included.

2. The second missing link is to understand how the buttock tissue deforms in response to the change of reaction forces when the seated posture is changed. This objective will also be investigated by a computational approach, using a commercially available FE-model of the buttocks (CASIMIR/Automotive). The model will be refined to fit the needs of this investigation, and boundary conditions will be generated by the musculo-skeletal model mentioned above.

3. The third missing link is a criterion relating deformation to cell death. This criterion should be based on experiments where cell cultures are subjected to different deformation states. It is important to notice that strain is a multidimensional property and controlling the strain felt by the individual cell requires an ingenious experimental setup. The included papers describe how cell cultures that can be stimulated mechanically by an indenter or by deforming a membrane the cells grow on, and computational methods are used to design experimental setups that allow detailed control of the strain state. The output goal of the subsequent experiments would be to correlate strain state and cell necrosis into a criterion quantifying ulceration risk for a given strain exposure.

4

Postural Effects

When sitting at a chair, the forces acting between the person and the chair depend on the sitting posture and the support surface, and they change if the chair and siting posture are varied (Bennett et al., 1984; Gilsdorf et al., 1991, 1990; Goossens et al., 1997; Hobson, 1992; Maurer and Sprigle, 2004; Roaf, 2006). Understanding how the forces are affected when the sitting posture changes is difficult due to many linked parameters. Many researchers have used an experimental approach and thereby provided useful information about how the forces distribute when the sitting posture changes (Gilsdorf et al., 1991, 1990; Goossens et al., 1997; Maurer and Sprigle, 2004). Even though the studies have been carefully executed, it remains difficult to compare findings because of experimental noise, statistical variations, and differences in protocols. Therefore a part of this project has been to use a computational approach to estimate realistic reaction forces for different seated postures. For this purpose, a seated musculo-skeletal model developed in the AnyBody Modeling System was used (Damsgaard et al., 2006). The seated model can predict reaction forces acting on the seated person, and also internal forces in joints and muscles. The predicted reaction forces can be used to understand how the forces change when the seated posture change, thereby relating seated posture and deformation of tissue. Any computational model should be validated with respect to the calculated predictions (Lund et al., 2012). To implement this, a validation of the reaction forces predicted by the seated anybody model has been carried out and documented in the following paper.

5

Paper 2: Experimental Validation of a Computational Seated Human Model for Pressure Ulcer Research

Experimental Validation of a Computational Seated Human Model for Pressure Ulcer Research

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ABSTRACT

Sitting-acquired deep tissue injuries (SADTI) are the most serious type of pressure ulcers. In order to investigate the aetiology of SADTI a new approach is under development: a musculo-skeletal model which can predict forces between the chair and the human body at different seated postures. This study focuses on validation of a model developed in the AnyBody Modeling System. A chair with force-measuring equipment was developed, an experiment was conducted with three subjects, and the experimental results were compared with the predictions of the computational model. The results show that the model predicted the changes in reaction forces due to changes in the chair posture well. The correlation coefficients of how well the experiment and model correlate for the seat angle, backrest angle and footrest height was 0.93, 0.96, and 0.95. It is concluded that the agreement with experimental data is sufficient to merit use of the model for prediction of forces between a human body and a chair. The model validated can in future be used in designing wheelchairs or automotive seats.

Keywords: Wheelchair, Musculoskeletal model, Pressure ulcer, Validation, Chair reaction forces

INTRODUCTION

Pressure ulcers, more commonly known as pressure sores, are a frequent complication to spinal cord injury (SCI) patients. The aetiology of the disease is in general poorly understood (Olesen, de Zee, & Rasmussen, 2010).

Statistics show that 24% of all patients with SCI experience a pressure ulcer during their rehabilitation hospital stay (Chen, Apple, Hudson, & Bode, 1999). It is also estimated that 50-85% of all patients with SCI will experience a pressure ulcer during their life time (Salzberg et al., 1996). These are very general prevalences covering a range of different types of pressure ulcers. The type of pressure ulcers that has motivated the present investigation is the sitting-acquired deep tissue injury (SADTI) that wheelchair users, i.e. paraplegic and quadriplegic patients are particularly susceptible to. The prevalence of deep tissue injury is difficult to assess because it is usually only detected after it has reached the skin surface, at which point the injury's origin is impossible to establish. SADTIs have a tendency to spread under the skin and reach proportions that are difficult to treat and in some cases terminal (Gefen, 2007).

Investigations of the aetiology behind pressure ulcers is broad and ranges from the seated posture over global loading of the buttocks to tissue stresses and strains and further on to cell deformation causing necrosis. It is well acknowledged that pressure ulcers are primarily caused by sustained mechanical loading of the soft tissues (Romanelli, Clark, Cherry, Colin, & Defloor, 2005; Schroeder, 2005). The types of loading can be described as pressure, pressure gradients and shear forces (Hobson, 1992). These loads conspire in a complicated fashion to generate deformation states varying from point to point in the soft tissues. An understanding of the input loads is therefore the first step towards a full understanding of pressure ulcer formation. The magnitude and position of the loads are influenced by the patient's posture and the support conditions of the wheelchair. Several research groups have contributed to the understanding of the forces between a seated human and its environment, but the difference in aims and experimental protocols make them impossible to compare (Bennett, Kavner, Lee, Trainor, & Lewis, 1984; Gilsdorf, Patterson, Fisher, & Appel, 1990; Gilsdorf, Patterson, & Fisher, 1991; Gilsdorf, Patterson, & Fisher, 1991; Goossens, Snijders, Holscher, Heerens, & Holman, 1997; Hobson, 1992; Maurer & Sprigle, 2004; Roaf, 2006). A recent review of the current literature within the field has pointed out the need to understand the forces acting on the buttocks for different seated postures (Olesen, de Zee, & Rasmussen, 2010). These forces can subsequently be used as global boundary conditions for FE models of the buttocks, which subsequently can calculate the internal strains and stresses in the tissue under the influence of various cushion materials. Several papers have described how the mechanics of the soft tissue on the buttocks react towards loading, but none have investigated how systematic change in posture change the deformation of the tissue. (Gefen, Gefen, Linder-Ganz, & Margulies, 2005; Linder-Ganz & Gefen, 2004; Linder-Ganz & Gefen, 2009; Stekelenburg, Oomens, Strijkers, Nicolay, & Bader, 2006)

A computational approach might contribute further to an understanding of how the seating posture affects the mechanical loading of the soft tissue in the buttock region. Such an understanding might also benefit designers of automotive, airline and office seats because of the contact forces' contribution to the perceived discomfort (Rasmussen & de Zee, 2009; Rasmussen, Tørholm, & de Zee, 2008).

A validated computational model could be used to predict the loading on the human body from different seated postures without the need for costly experiments. Furthermore, an analytical model is free from the inevitable experimental noise and statistical variations due to individual differences between subjects and differences in protocols that may otherwise occlude the interpretation of results.

However, before using any model it should be validated with respect to the predicted forces (Nigg & Herzog, 1999). Therefore the objectives of this study are to validate a musculo-skeletal model with respect to its ability to predict the chair reaction forces and how these are influenced by changes in the seated posture. This is accomplished by comparing force predictions from a model with measurements from test subjects.

METHODS AND MATERIALS

The study design comprised an experimental and a modelling part. The experimental setup included measurements of reaction forces on a wheelchair measured in synchronization with motion capture data for recording of the sitting posture of three volunteers. A musculo-skeletal model was created to mimic the seated position and thereby estimate reaction forces between the chair and the model. These reaction forces could then be compared with the forces measured during the experiment and in that way validate the musculo-skeletal model. For a study overview, see figure 1.

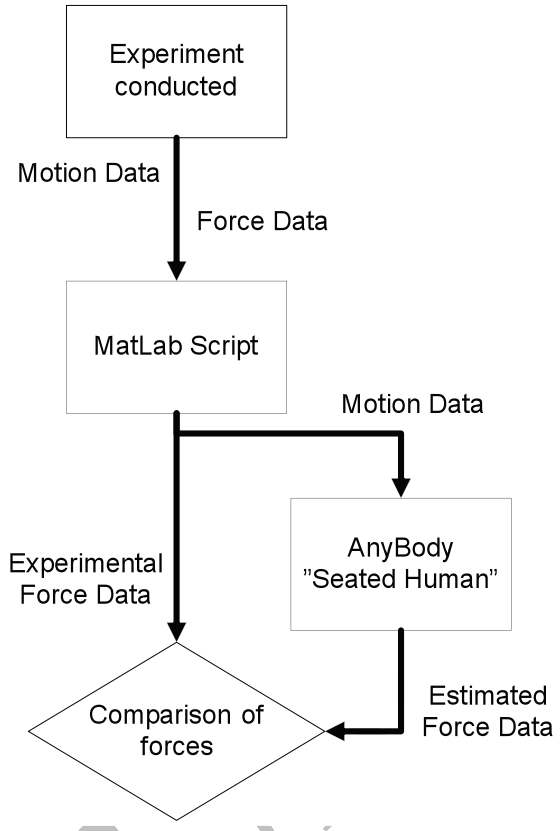

Figure 1 Flow chart of the study design. Recording and post processing of experimental data using a Matlab script, transfer of the kinematic data to the musculo-skeletal model for simulation of forces and finally comparison between measured and calculated forces.

Experimental Setup

Force measurements

A custom-built wheelchair, see figure 2 (Wolturnus A/S, Nibe, Denmark) was mounted with force-measuring equipment (Advanced Mechanical Technology, Inc., Watertown, MA, US). The chair was constructed by mounting an OR6-7-1000 force plate as a seat, as a footrest an OR6-7-2000 force plate mounted in the floor was used. The backrest consisted of two horizontal bars that in each side were mounted to two multi-axis force and torque transducers. In total, four multi-axis force transducers (2 x FS6-250 & 2 x FS6-500) were mounted in the two backrest bars. The backrest bars were covered with 4 mm foam and the seat was covered with a 2 mm rubber mat.

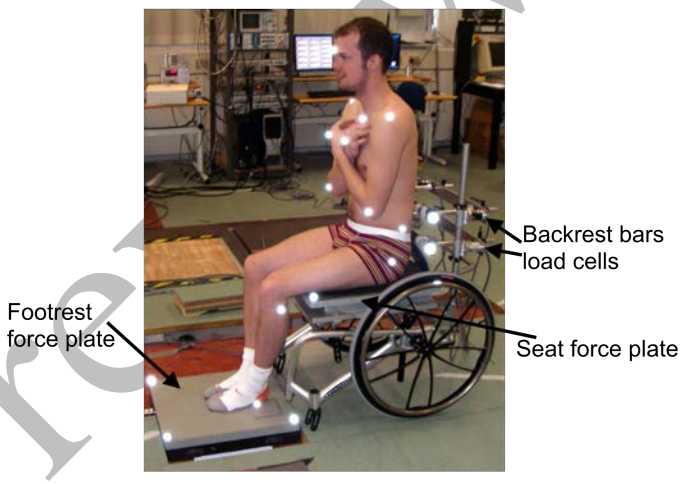

Figure 2 The experimental setup with a subject sitting on the instrumented chair. The white dots are the reflective markers used for motion capture.

The wheelchair could be adjusted in a number of ways. The inclination angle of the seat and backrest could be changed. The seat could slide forward/backward. The two backrest bars could individually slide up/down and forward/backwards. These adjustments allowed the wheelchair to be adjusted continuously into any position with respect to backrest height, seat depth, seat angle and backrest angle. The forces applied to the force plates and force transducers were amplified using amplifiers from the same manufacturer as the force measuring equipment. The amplified signals were sampled using Texas Instrument 16 bit A/D converters, connected to a computer equipped with data collection software (Mr.Kick, Aalborg University, Aalborg, Denmark) (Larsen). The six channels on each force-measuring device represented the 3 forces and 3 moments, they were all sampled giving a total of 36 channels. The sampling rate was

20 Hz over 10 seconds giving 200 samples per channel.

Posture Assessment

The chair positions were measured using a motion capture system (Qualisys Proreflex 240, Gothenburg, Sweden) with eight cameras. Passive reflective markers were placed in each corner of the two force plates and on both sides of the two backrest bars. The posture of the subject sitting in the wheelchair was also measured using passive reflective markers. The markers were placed at the following anatomical landmarks: Forehead (glabella), sternum, the lateral tip of the acromion, the lateral elbow epicondyle), hand (Os sesamoideum of digitus tertius), pelvis (Crista iliaca), greater trochanter, knee (Caput fibulae) and lateral malleolus.

The positions of the markers were measured with a sampling rate of 20 Hz over 10 seconds.

The force measurements and the motion capture system were started by a trigger in order to synchronize the force and motion capture recordings.

Conducting the Experiment

Three able-bodied men of 179±3cm height and 68±2 kg body mass were included in the study.

The subjects' height, mass and height from floor to the posterior side of the knee while seated were measured. The posterior knee height was used as a guide for an appropriate seat height, i.e. the distance from the footrest force plate to the front top edge of the seat. This distance was kept constant throughout all experiments, except for the experiment varying the seat height. The subject was seated in the chair and the markers were attached. The subject was instructed to sit as relaxed as possible with the arms crossed over the chest in order to make sure they did not affect the result. The force plates and load cells were reset every time the posture of the chair was changed to make sure that the baseline was the same for all measurements. The experiment included 12 different seated postures.

The seat angle was varied from -17° to 6°, while the backrest angle was maintained 18° leaned back, and the footrest height adjusted relatively to the subject's lower leg length. The backrest angle was varied from approximately 90° corresponding to vertical, and reclined to approximately 123°. The seat was kept in 7° leaned backwards, and the footrest height adjusted relatively to the subject's lower leg length. The distance from the edge of the seat to the footrest varied from lower leg length plus 2 cm to lower leg length minus 8 cm. while the backrest angle was maintained 18° leaned back, and the seat angle was kept 7° leaned back. Between each of the posture variations, only one parameter was changed and the subject sat in the chair 4 minutes between each measurement to ensure that the seated position had reached a steady state as described by Crawford, S.A. et. al. (2005) The continuous adjustments and the fitting of the seat to the different anthropometries of the test subjects meant that different postures were recorded for the three subjects.

Post Processing

Motion Capture

The motion capture data from the Qualisys cameras were initially post processed in QTM (Qualisys

Table 1 The seat angle, backrest angle and footrest height for the different experiments. The diagonal of ranges were the parameters changed in each of the experiments

	Seat angle	Backrest angle	Footrest height
Seat angle variation	-17°- 6°	18°	H*
Backrest angle variation	7°	90° - 123°	H*
Footrest height variation	7°	18°	(H*-8cm) – (H*+2cm)

Tracking Manager V.1.10.282) where the markers were identified and named. The markers were then exported to a TSV file and then processed using a custom-made Matlab (MatLab R2008B, Mathworks Inc., Ma, US.) script that calculated the angles and positions between the seat, backrest bars and footrest. The output of the Matlab scripts was a text file that could be included in the AnyBody model. The text file contained all the marker positions for driving the model. For an overview of the process, see **Fejl! Henvisningskilden blev ikke fundet.**1.

Force Data

The collected force data was gathered using Mr. Kick saving each data file as a Matlab data file. A script was used for averaging the values. A Matlab script combined the motion capture data with the measured forces.

Musculoskeletal model

The musculoskeletal analysis was performed in the AnyBody Modeling System version 4.1 (AnyBody Technology A/S, Aalborg, Denmark). The musculoskeletal model is based on the "Seated Human" model from the open source AnyBody model repository (AnyScript Community), which was described in (Dahlquist, Christensen, Rasmussen, Zee, & Damsgaard, 2004; Rasmussen & de Zee, 2009). The AnyBody Modeling System is computer software designed for constructing musculo-skeletal models of the human body and its environment and for determining how these interact. With this kind of models it is possible to estimate muscle activities, joint reaction forces and unknown interaction forces with the environment using optimization algorithms. The mathematics and mechanical theories behind it were described by (Damsgaard, Rasmussen, Christensen, Surma, & de Zee, 2006).

The model was scaled in mass to the body mass of each test subject. The subjects were approximately the same anthropometrical size as the generic model in the public repository,

The model consists of two parts: a human model and an environment representing the wheelchair. The seated model relies on a set of assumptions described in (Rasmussen, Tørholm, & de Zee, 2008). In addition, a few assumptions were made about the interface between the chair and the human body:

Contrary to real seated persons, rigid multibody models are by nature supported on points rather than surfaces and by reaction forces rather than by pressure distributions. The model has therefore been equipped with a number of points through which it can transfer reaction forces to the supporting elements, i.e. the backrest, seat pan and footrest. The contact between the body and the supporting elements is modelled by means of contact elements that can provide only compressive reactions and shear forces implemented as Coulomb friction in the contact points. The friction coefficients μ, between the body and the seat and footrest was estimated to $\mu=0.5$ because the surface material was rubber. For the backrest $\mu=0.1$ was used because the foam wrapped about the dynamometers could rotate.

Comparison

Comparing absolute force values and trends when changing an input parameter did the comparison between the experimentally obtained forces and the estimated forces from the musculoskeletal model. The force values were plotted as a function of the variable parameter. The forces used for comparison were the seat shear forces and the normal force on the footrest. The forces on the backrest relates directly to the forces on the seat, therefore these were not reported. The normal force of the seat is also an important parameter when looking at pressure ulcer research, however it does not vary much when changing posture, due to the big contribution from gravity.

RESULTS

The results from the forces measured during the experiment and the estimated forces from the musculoskeletal model were compared as absolute values and trends of these while changing one parameter at the time.

Effect of changing the backrest angle

Figure 3 illustrates the experimental and model results for the three subjects for the backrest angle variation. The results indicate that backrest

inclination increases the seat shear force. Linear regression lines for each of the datasets reveal that the slopes differ by 18.8 %. This corresponds to an absolute difference of maximally 20 N at the two extremes because the two curves intersect very closely to 0N. Please notice, however that despite the addition of the linear regression lines, nothing in the physics of the problem indicates that the behaviour should be linear. The correlation coefficient, R^2-value and confidence interval of how well the model describes the experiment can be found in table 1.

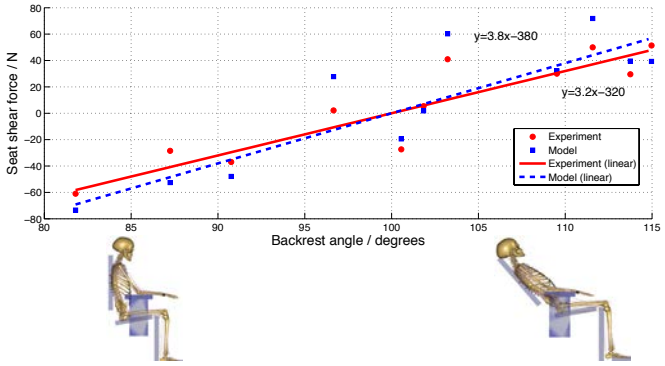

Figure 3 The effect on the seat shear force from varying the backrest angle. The data show clear correspondence and a trend towards higher seat shear force when the backrest is inclined.

Effect of changing the seat angle

Figure 4 shows that changing the seat angle from tilting forward to tilting backwards while keeping the other variables constant has a strong influence on the seat shear force. In figure 4. experimental and modelling results have been pooled for the three subjects, and a linear regression line has been plotted for each of the datasets. The slopes differ by 19.8 % leading to a maximum deviation between the linear regressions of experimental data versus model data of approximately 20 N. The correlation coefficient and confidence interval for the experimental and model of the seat angle can be found in table 1.

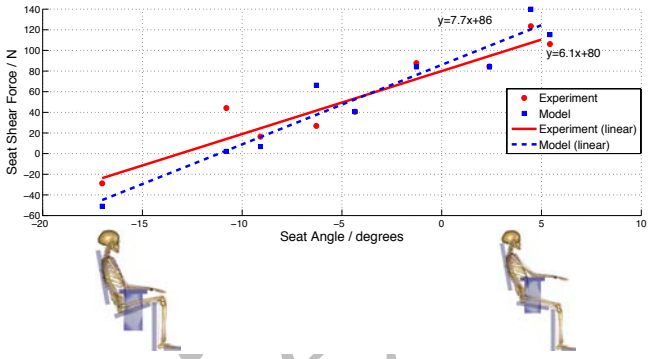

Figure 4 The effect on the seat shear force when the seat angle is changed. The data show clear correspondence and similar trends can be observed as well.

Effect of changing the height of the footrest

The height of the footrest has an effect on the seat normal force predicted by the model. Figure 5 shows the difference between the measured and the predicted normal force on the footrest. Most of the data points are within ±20N and the standard deviation of the error is 15.5 N. The correlation coefficient and confidence interval for the experimental and model of the footrest height can be found in table 1.

Table 2 The correlation coefficients, the confidence interval and the RMS error for the seat normal and shear force prediction for each of the three experiments.

	Seat angle	Backrest angle	Footrest height
Correlation coefficient	0.93	0.96	0.95
R^2-value	0.87	0.91	0.91
95% Confidence interval	0.71-0.99	0.84-0.99	0.82-0.99
RMS Seat Fs Error	20.0 N	13.6 N	13.7 N
RMS Seat Fn Error	22.3 N	38.4 N	35.4 N

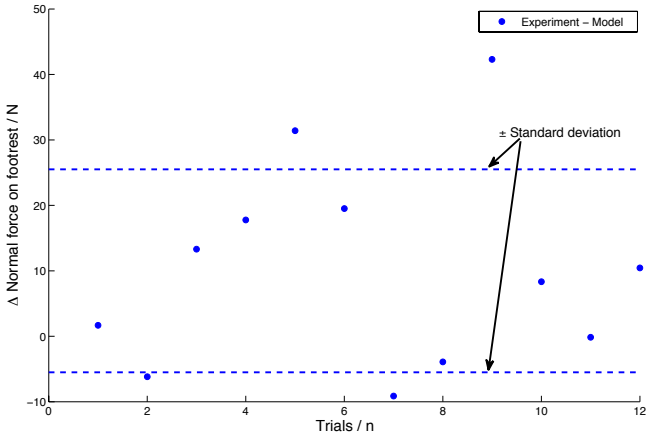

Figure 5 The difference between the measured and predicted normal force on the footrest. The different data points are for the different experiments where the height of the footrest was changed.

DISCUSSION

The results quantify the correspondence between the computational musculoskeletal model and the experimentally obtained data. It was found that the seat shear force and the footrest normal force were the ones that varied the most when changing posture, the other forces and moments did not vary much not in the experiment, nor in the model predictions, therefore these forces were the main focus point in the result section.

Varying the seat angle, backrest angle and footrest height in general showed good results and the model predicted the forces acting between the chair and the subjects. In each experiment the goal was to vary a single parameter, but due to (i) the continuous adjustment of the chair, (ii) the fitting of the chair to each subject's anthropometry, (iii) the small postural adjustments of the subject in each trial and (iv) the different choices of posture in a given chair setup between the subjects, there was small deviation in the chair setup and realized posture between each of the experiments. However, the majority of this variation was transferred to the model via the kinematic input from each of the experiments to the model.

Other researchers have conducted experiments that can be compared with the present results. For example Gildorf, P. et al. (1991) mounted a force plate as a wheelchair seat and measured normal and shear forces for different backrest angles. They found the average shear force at the seat for a hard surface at 5˚ incline to be 27 N, which corresponds well with this study, indicating that the test subject was typical. In another study conducted by Bush, T.R. & Hubbard, R.P. (2007), 12 healthy mid-sized males were seated in an instrumented chair in different seated postures, and normal and shear forces were measured. The experimental setup was somewhat different, but approximately 15 N seat shear force was measured in a neutral posture with the backrest reclined 20 degrees. This could be compared with the graph on figure 3, predicting between 10 and 20 N seat shear force for the same backrest angle.

Also Hobson, D.A. (1992) did experimental work on different seated postures on spinal cord injured and able-bodied subjects. Their results are difficult to compare, since the protocol used in this study, is not similar, however some comparison can be made between seated postures where there are only a few degrees difference in seat- backrest angle. Posture "P4" where the seat is horizontal and the backrest is leaned back to 110˚ is somewhat comparable to the seat angle variation experiment in this study. Hobson, D.A. measured a mean 70 N shear force, where the experimental results from this study was 75-80 N.

The purpose of the seated musculo-skeletal model is to give detailed information on how a change in seated posture affects the reaction forces between the chair and the human body. It is a model of how parameters interact trend validation is an important measure of model quality(Nigg & Herzog, 1999). The correlation coefficient between predicted and measured shear force with backrest angle variation was 0.9580 with a 95% confidence interval between 0.8419-0.9893 indicating that with 95% certainty the model can describe at least 84% of the experimental result. Similar results were found for variation of the footrest height, while the correlation coefficient for variation of the seat angle was 0.9335. Most likely, with more experiments, the confidence interval would become smaller and thereby the certainty of how well the model describes the experiment would be larger. The R^2 values for the 3 experiments show

that the difference between the experimental and modelling results were relatively small.

The experiment was conducted with three subjects with a normal body mass index, if for example one of the subjects had been over or under weight the model would have to be scaled in order to fit the test person. However the study was focused on validating the model as is, not validating the scaling methods, hence the small variation between the three subjects.

The results from varying the footrest showed a bias towards the model predicting lower forces than measured in experiments, the difference is not big, however most predictions were lower than the matching experimental data. The largest error seen on figure 5 is when the footrest is raised, i.e. the distance from the footrest to the seat is small. This could be explained by the passive elastic forces there is in the body when the thorax/thigh angle becomes smaller, which is not build into the AnyBody model. The subjects lower legs were 48-50 cm therefore the most important part of the graph is around this height, where the errors were small. The large errors seen on the figure comes from somewhat unnatural sitting postures.

The model predicted reaction forces between a chair and an able-bodied person, and therefore muscles were included in the model. The muscle activities calculated by the model were low in all the modelled postures (< 4%). There was, however, a slight increase in the estimated muscle activation when the friction coefficient decreases, indicating that in these situations muscle activity is necessary to avoid slipping from the chair.

There were a couple of possible error sources in the experiments, such as marker placement, which could cause prediction errors. The chosen friction coefficients for the seat and footrest were investigated in a sensitivity study where the two coefficients where varied and the shear force at the seat estimated by the model was the output. The model turned out to be insensitive to the friction coefficients if they were above μ=0.15. Thus, if the true friction coefficient if above 0.15, the assumed coefficient of 0.5 does not influence the result.

The study included experimental results from three subjects, that were chosen to match the standard AnyBody model, this was done to exclude scaling, because this study was done in order to validate the model, not the scaling method, which should be addressed in future studies,.. For future patient specific cases, it will be essential to scale the model to the subject. Especially for disabled subjects, segment masses may have atypical values and some muscle groups should be remodelled.

The musculo-skeletal model estimates reaction forces between the chair and the human body with the same trends that can be measured experimentally, and also the absolute values correspond quite well. Overall the result is encouraging with respect to the opportunities to use a computational model for seat adjustments aimed at controlling the reaction forces in the human body. The resulting forces can be used as boundary conditions for a finite element model of the buttocks for prediction of soft tissue strains in pressure ulcer research and in improvement of seat comfort in general.

ACKNOWLEDGMENTS

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6

Buttocks Tissue Deformation

As mentioned in a previous chapter, cell deformation is believed to be the underlying reason for pressure ulcer initiation. The local state of deformation is described by the strain tensor, which is a multidimensional property depending in a complex fashion on the boundary conditions of the problem. The boundary conditions for the soft tissue are influenced by the reaction forces caused by the seated posture but also by the complex geometry of interfaces between the different tissue types, for instance the shape of the pelvic bone. Furthermore, strains are influenced by the complex material properties and large deformations of soft tissues leading to a highly nonlinear behavior. Strains therefore escape an intuitive understanding, and it is not given, for instance, that external shear forces between the seat and the buttocks also directly lead to shear strains in the soft tissue. A finite element model (FE-model) can be used to predict internal strains as a function of external boundary conditions. A FE-model consists of three parts. The first part is the geometry of the model, which in this case is the anatomy of the human buttocks and the seat. The second part is the constitutive properties, i.e. the material properties linking strains with stresses. The final part of the FE-model is boundary conditions, i.e. the forces acting between the buttock tissue and the chair.

All of these elements are complex to identify and develop, and therefore it is decided to build upon an existing and carefully developed model rather than creating a one from scratch. The FE-model used in this project was developed by Wölfel Beratende Ingenieure GmbH for use in the automotive seat industry. The model has been described and validated in several publications (Pankoke et al., 1998; Siefert et al., 2010, 2008). Therefore, the geometry and material properties have been adopted from the commercial model and have not been considered in this project. The boundary conditions come from the seated AnyBody model, presented and validated in chapter **??**. The two types of models (FE-model and AnyBody model) in combination can answer research questions none of them can independently: the relationship between the seated posture and the tissue strain over bony prominences where pressure ulcers are suspected to initiate. This relationship can be used to design wheelchair mechanisms controlling the combinations of seat/backrest angles that minimize the tissue strain. This has

been demonstrated in the following paper investigating the effect of using the "Tilt-in-Space" function that many wheelchairs offer.

7

Paper 3: Relationship between tilt in space and risk of pressure ulcer development

Buttock strain estimation in tilt-in-space wheelchair users by musculoskeletal modeling and 3-D finite element models

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ABSTRACT

This study investigates interactions between seat reaction forces and the buttock tissue strain in users of wheelchairs with a tilt-in-space function. The study comprises a musculo-skeletal computational model estimating chair reaction forces, and a finite element model of the buttocks estimating tissue strain as a function of the tilt angle. The results show that the function relieves normal forces on the buttocks at the cost of increasing the seat shear force. The changed forces cause a decrease in maximum shear strain, but not in maximum normal strain. The results show that the relationship between reaction forces and tissue strain under the buttocks is rather complex. Therefore it is important to further investigate relationship between chair reaction forces and tissue strains.

Keywords: CASIMIR, AnyBody, Pressure ulcer, FEM, buttocks

INTRODUCTION

Pressure ulcers affect the quality of life of wheelchair users (17), and it is the most common cause of rehospitalization for people with spinal cord injury (SCI) (13). Studies have shown that more than 80% of wheelchair users will develop a pressure ulcer at some point in their lifetime (21). Bennett et al. (3) estimate that the cost of treating one pressure ulcer in the UK National Health Service (NHS) ranges between £1,064 and £10,551 (€1,250 – €12,398) and that the total costs for this treatment comprise 4% of the total NHS budget, i.e. in the billion Euro range for the UK alone.

The current knowledge on pressure ulcer etiology in general has been formulated into a comprehensive book by Bader et al. (2). 'Pressure ulcer' is a general term that is widely used about tissue damage from superficial heel sores to deep buttock necrosis, the latter being a so-called deep tissue injury, which is considered to be especially harmful as the tissue layers between the skin and bone may suffer substantial injury (1). There is an agreement that sitting-acquired deep tissue injuries (SADTI) are due to necrosis under the buttocks and the initial cause is mechanical deformation of tissue (1). The mechanical deformation is caused by reaction forces acting between the chair and the tissue of the person sitting in the chair. The reaction forces change considerably when the seated posture or wheelchair adjustments are being varied (5, 10, 11, 15) and seated posture therefore influences the risk of ulceration.

A particularly common wheelchair adjustment capability is called Tilt-in-Space. In this adjustment, the entire chair (backrest, seat, and footrest) is leaned back while the angles between the parts are kept constant (16). The Tilt-in-Space function is popular among wheelchair users (24) and has been shown by means of pressure measurements devices to effectively decrease interface pressure in the buttock area (8), which most likely is due to the change in gravity direction with respect to the seat surface. However, the interface pressure measurements do not register shear forces parallel to the seat even though these forces are also affected by the Tilt-in-Space angle and affect the tissue deformation under the buttocks.

Linder-Ganz et al. (14) combined seated MRI scans using a double-donut scanner with a 2-D Finite Element (FE) modeling approach and found large deformations in the tissue under the buttocks of healthy and paraplegic individuals on a semi-rigid seat. The combination of MRI scans and FE modeling is an ideal approach because it allows for analysis of deformations resulting from actual observed boundary conditions. However, the necessary double-donut scanners are rare and not easily accessible and a 2-D FE model does not represent the three-dimensional nature of the geometry very well. Thus, knowledge of how reaction forces between a person and a chair depend on posture and how they are transformed into tissue strain is still missing (19).

Therefore the objective of this study is to investigate how the Tilt-in-Space functionality affects the buttock tissue strain by means of a 3-D FE-model with realistic boundary conditions provided by a validated musculo-skeletal model.

METHOD

The study involves two different mechanical models, a musculo-skeletal multi-body model, see figure 1a, and a FE model, see figure 1b. The musculo-skeletal model is capable of computing

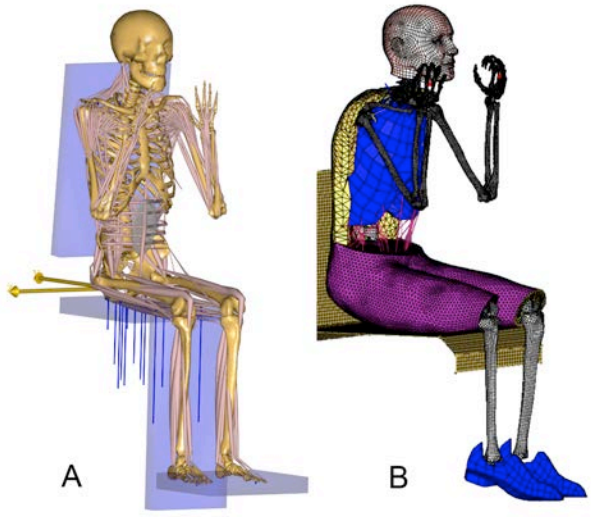

Figure 1 A: The AnyBody Seated Model used in the study. B: The CASIMIR/Automotive seated FE model used in the study.

the normal and shear forces between the human and the chair, and it feeds these forces as boundary conditions into the FE-model.

FE model

The FE model (fig. 1b) is the commercially available CASIMIR/Automotive model (Wölfel Beratende Ingenieure GmbH, Höchberg, Germany). The model has been developed for the automotive seating industry. Several publications describing the model and the validation of the model are available (20, 22, 23). The model is preprocessed in HyperMesh (Altair Engineering Inc. Troy, MI, USA) and exported to an input file for Abaqus vers. 6.10.4 (Simulia, Rising Sun Mills, Providence, RI, USA). The model includes bones, soft tissue, muscles, and ligaments. For the purpose of the present work, the buttock part of the model (pelvis, femur, tissue and skin) was remeshed to a finer grid in order to predict local strains under the ischial tuberosities more precisely. The buttock tissue surrounding the femur and pelvis has been split into two different types of tissue, consisting of an upper and lower part. In total the tissue includes 61,732 nodes and 285,259 tetrahedral elements with a nine times higher element density under the ischial tuberosities compared to the original model. The material model used for the tissue was a non-linear hyperelastic Mooney-Rivlin model. The buttock FE model also included a rigid seat where contact between seat and buttocks was defined with friction coefficient μ=0.4, as earlier used for validation purposes of the musculo-skeletal model. (18).

Rigid body model

The rigid-body musculoskeletal model (fig. 1a) was developed in the AnyBody Modeling System (AnyBody Technology A/S, Aalborg, Denmark) and was set up to simulate a person sitting in a chair using the Tilt-in-Space function. The seated AnyBody model is comprised of the skeletal bones, muscles and ligaments. The model is sitting in a chair with seat, backrest and footrest. Contact was defined between the body and the chair. The model posture is set up and solved with respect to the internal and external forces, meaning that the global forces acting between the chair and the body were calculated, taking into account posture and internal muscle recruitment. The model has been experimentally validated with respect to the predicted forces (18). The simulations made with the AnyBody model are illustrated in figure 2, where the model is visualized with the different tilt-in-space angles. The estimated forces are illustrated in figure 3 as reaction forces, and the magnitudes are listed in table 1.

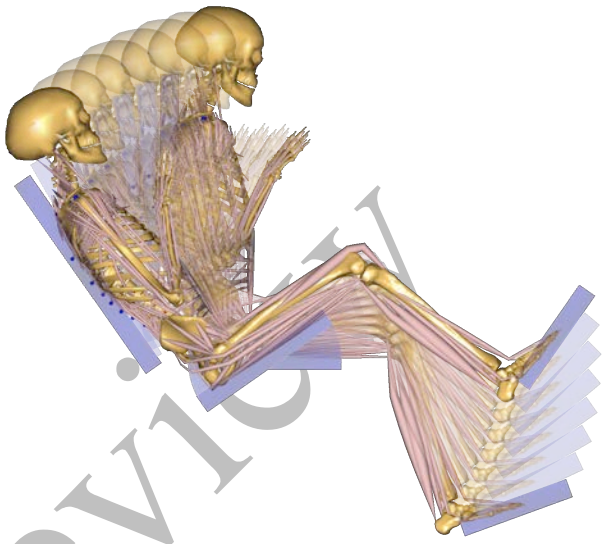

Figure 2 The AnyBody Seated Model Tilt-in-Space movement.

Tilt-angle	Seat Normal f	Seat Shear f
0	777 N	23 N
5	765 N	5 N
10	750 N	-15 N
15	727 N	-39 N
20	699 N	-62 N
25	669 N	-85 N
30	638 N	-106 N
35	601 N	-123 N

Table 1. The normal and shear forces predicted by the AnyBody seated model for the different tilt-in-space angles.

Strain estimation

The FE model of the buttocks was used to estimate strain states in the tissue under the ischial tuberosities after application of the posture-dependent forces. The human model was placed in contact with a seat. The pelvis and femur were constrained in all six degrees-of-freedom and the seat was translated in the z-direction until

the AnyBody model-estimated normal force was reached. In the second step the estimated shear force was applied to the seat forward or backwards, depending on the predicted force direction.

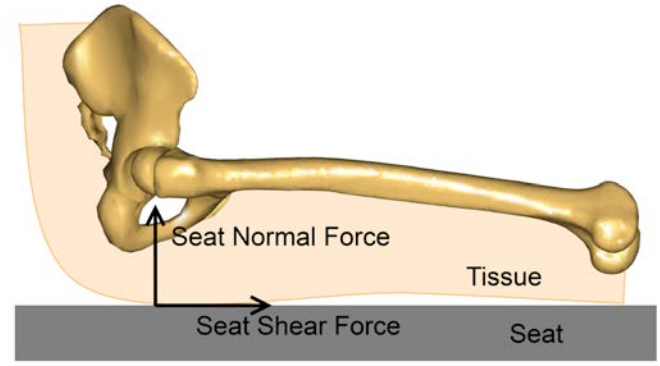

Figure 3 An overview of the force directions related to the buttocks.

When the boundary conditions were set, the maximum shear strain was calculated for each element from the principal strains.

$$\tau_{\max} = \frac{\varepsilon_1 - \varepsilon_3}{2}$$

where $\boldsymbol{\varepsilon_1}$ and $\boldsymbol{\varepsilon_3}$ respectively are the maximum and minimum principal strains.

The maximum shear strain for each element was exported and ranged from highest to lowest. The peak and mean values and 95th and 99th percentile were reported in the result section.

RESULTS

The FE-model was solved with the boundary conditions from table 1. The solved model is visualized in figure 4, where the compressive strain is plotted in a parasagittal plane intersecting the buttock.

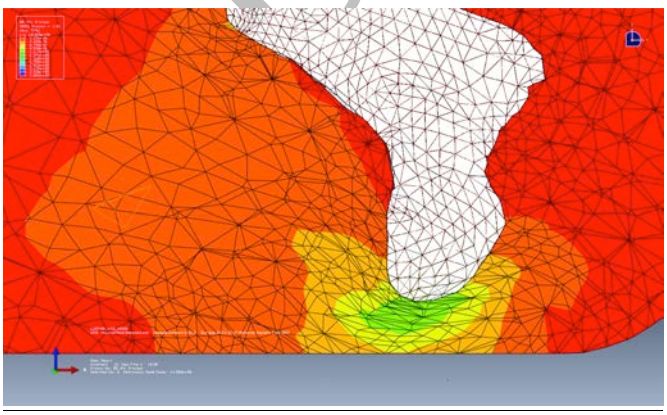

Figure 4 A magnified view of the maximum compressive strain under the ischial tuberosities of the buttock model. The white part is the inferior aspect of the pelvis.

The peak compressive strain appears under the ischial tuberosities.

The maximum compressive and shear strain in the buttock region was calculated for each of the different Tilt-In-Space angles and the results were plotted in figures 5 and 6. The graphs in figure 5 show that the peak values for the maximum compressive strain vary as the seat is tilted backwards, but there is no obvious trend following the Tilt-In-Space angle. However the 99th and 95th percentiles do show a slight trend towards less strain when the chair is tilted.

Figure 6 shows that peak and 99th percentile of the maximum shear strain decrease slightly as the tilt angles increase, while the 95th percentile remains almost constant.

DISCUSSION

The musculoskeletal model estimated (Table 1) that the Tilt-in-Space function decreases seat normal force, which is in agreement with experimental results (8). However, the model also shows that the shear forces simultaneously increase, which is new knowledge that cannot be obtained with pressure mats.

The FE model using the predicted forces as input shows that the decrease in normal force obtained with the Tilt-In-Space function does lead to a significant decrease of maximum compressive strain under the ischial tuberosities but, despite the increase of shear force, no increase of maximum shear strain under the ischial tuberosities can be observed. In fact, the combination of changes imposed by the Tilt-In-Space function decreases the maximum shear strain moderately. These complexities are caused by the fact that, in continuum mechanics, the principal strain directions change with the local state of deformation and therefore do not relate in any simple way to the normal and shear directions defined by the seat orientation. The combined method of a musculo-skeletal model and a 3-D seated finite element model seems to make it possible to manage the complex relationship between the external forces on the body and the strain state in the tissue while changing the seating posture.

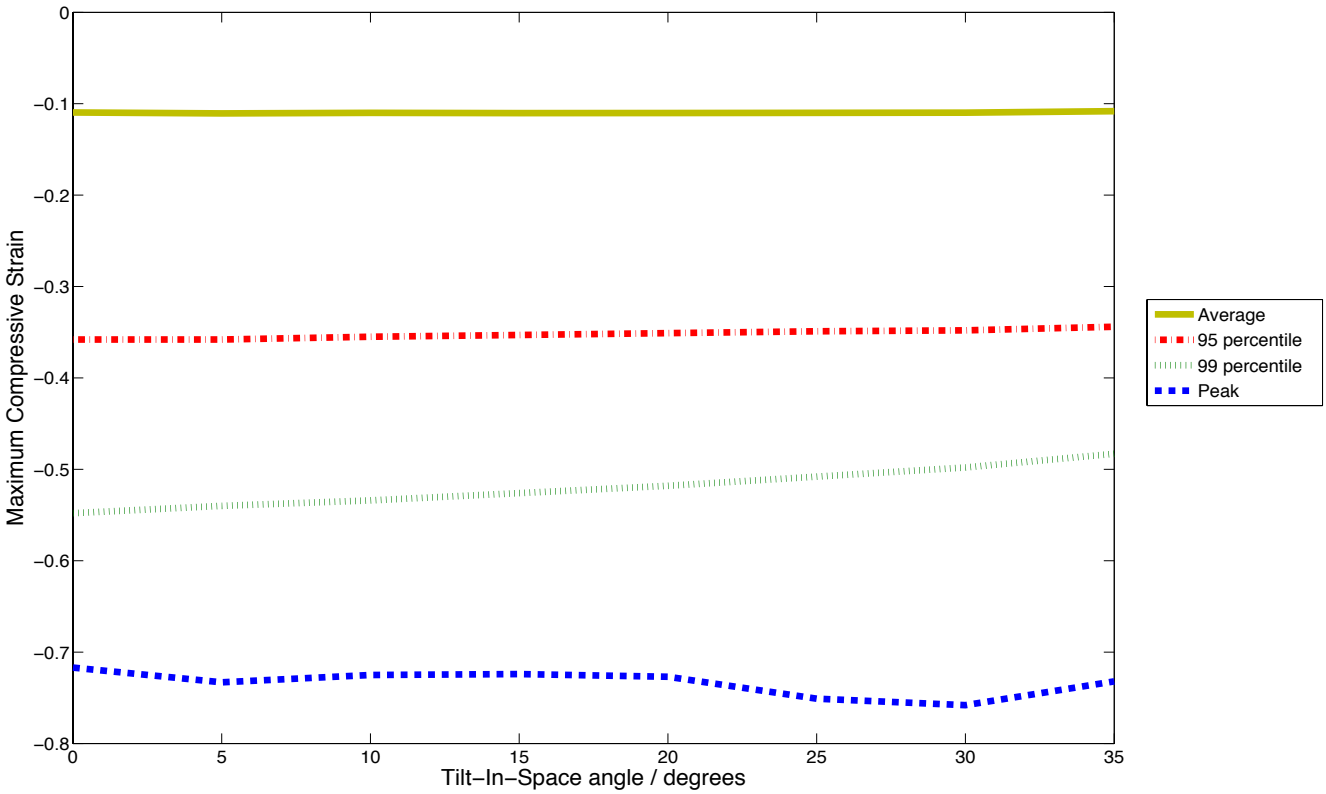

Figure 5 Statistical processing of nodal compressive strains, i.e. third principal strain, as a function of the Tilt-in-Space angle. The Tilt-in-Space angle has little influence but reduces the 99th percentile slightly.

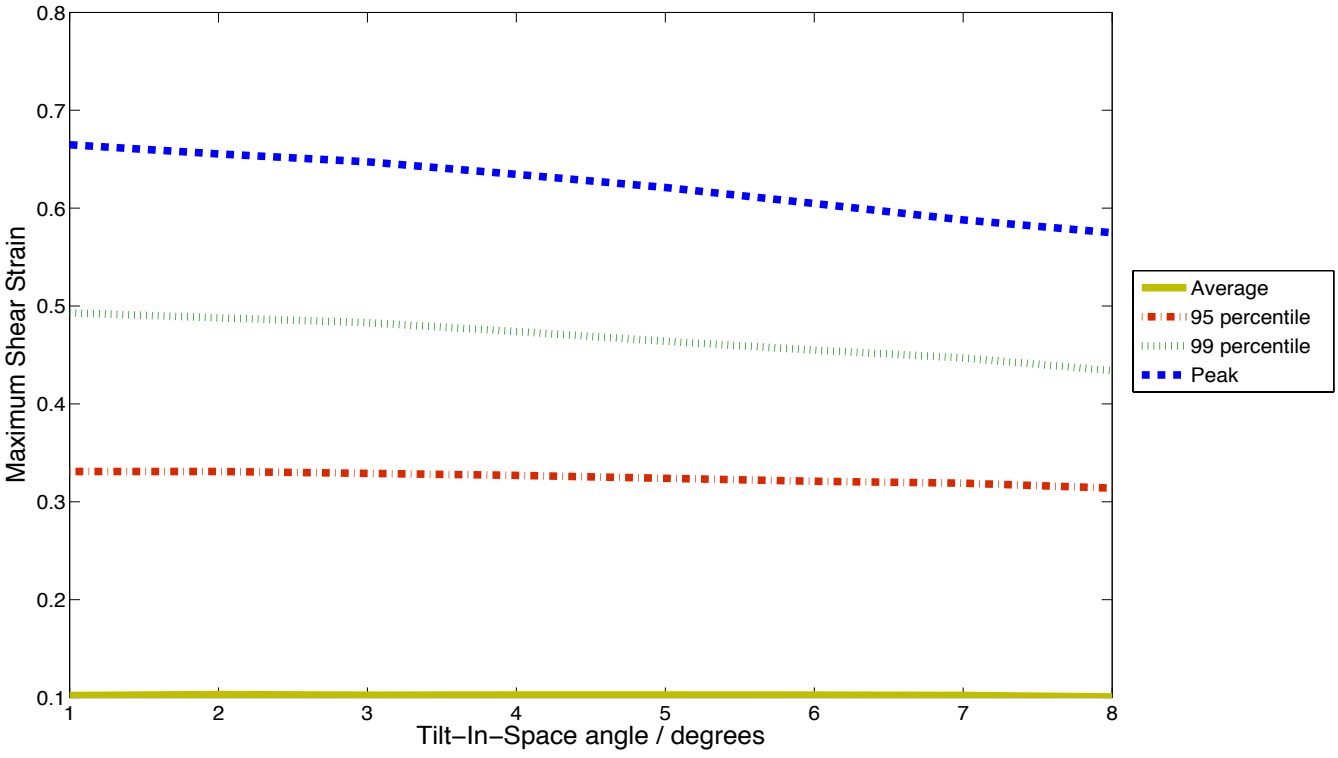

Figure 6 Statistical processing of nodal shear strains as a function of the Tilt-in-Space angle. The Tilt-in-Space angle has no influence on averages but progressively reduces the higher percentiles.

Several studies describe shear strain as a main reason for tissue necrosis (4, 6, 72). Given the demonstrated decrease of peak shear strain in deep tissues it can be argued that Tilt-In-Space decreases the risk of de
ep tissue injury. However the tilt does increase seat shear force and studies have proposed the seat shear force as a risk factor for pressure ulcer development (9, 10), thus indicating that tilted chair angles will entail an increased risk of superficial initiation of a pressure ulcer.

The apparent trade-off between superficial and deep tissue loads in the tilt-in-space function leads to the suspicion that postural relationships offering a larger reduction of both terms may exist. Given the considerable computation times of the nonlinear finite element analysis, a systematic identification of the optimum relationship would be comprehensive endeavor and is beyond the scope of this article. However, since the musculoskeletal model is fairly computationally efficient, it is feasible to conduct a parameter study of the seat pan shear forces for all combinations of seat pan versus backrest angles as illustrated in figure 7. The parameter study clearly identifies a relationship, illustrated by the dotted curve in the figure, for which the seat shear force is theoretically zero, thus possibly reducing the risk of superficial ulcers. Future work will reveal whether this or other postures also reduce the deep tissue strains.

CONCLUSION

The present work shows that the combination of rigid-body musculoskeletal models and FE models of the soft tissues can be a feasible way of investigating the relationship between seated posture and tissue strain. Given the absence of a physically obvious relationship between posture

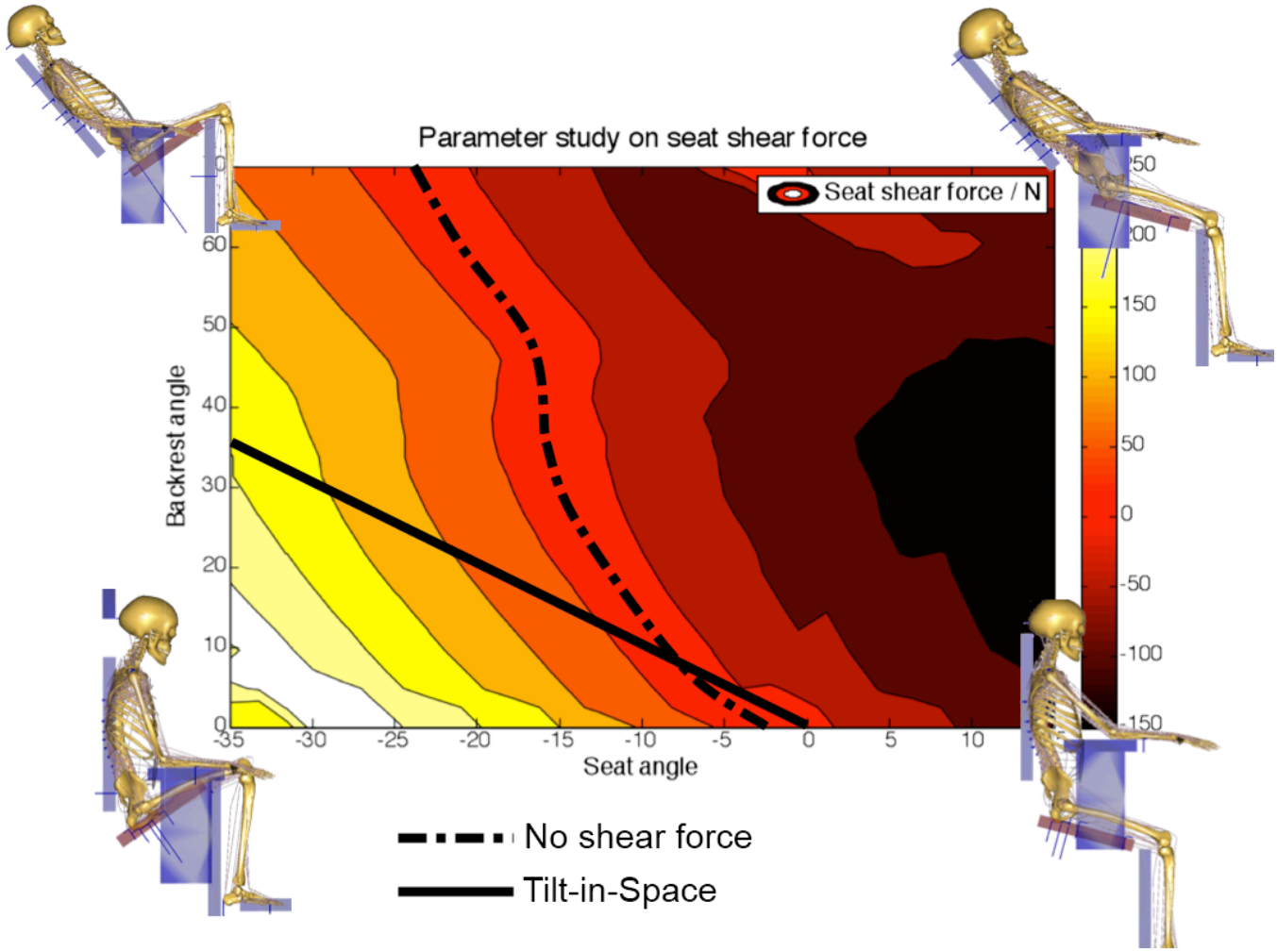

Figure 5 Parameter study of the influence of posture in the musculoskeletal Seated Human model with the backrest and seat angles on the vertical and horizontal axes respectively. The four figures in the corners illustrate the extreme postures. The color levels represent the seat shear force, and the dashed line represents the combinations of seat and backrest angle where the seat shear force is zero. The solid line represents the backrest and seat angle combinations obtained with the Tilt-in-Space function.

and shear strains near the ischial tuberosities it is not surprising that therapists struggle to intervene successfully in pressure ulcer prevention and rehabilitation cases.

The work is still in an early phase with obvious limitations. One limitation is the fact that the FE-model sits on a rigid plate, which is different from a normal wheelchair seat pan and is likely to influence the absolute values of the strains and, according to the principle of Saint Venant, primarily in the superficial regions. Another limitation is that the present models are based on data from able-bodied individuals, which may be quite different from typical wheelchair users. In future work, the present generic model can be modified to better represent a variety of individuals. Finally, the soft tissue model does not take the actual muscle configuration, the anisotropy of the muscle tissue and the stiffness differences between different soft tissues into account, and this may also influence the simulated strain state.

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8

Cell Death Criterion

In this chapter, a cell death criterion means a relationship between the strain state and possibly time of exposure, and the risk of subsequent necrosis of the cell. Although it was pointed out in chapter **??** that deformation alone seems to be the principal cause of observed cell necrosis, the actual mechanisms leading from cell deformation to apoptosis and subsequent necrosis are not understood. Therefore it is also at present impossible to obtain an understanding of the problem by purely computational approaches; experimental investigations are required, but their design can be significantly enhanced by computational model, and this is the topic of the present chapter.

The first step is to develop a method to subject cell cultures to controlled mechanical stimulations. In this project, the commercially available Flexcell system was used. The Flexcell system consists of flexible membranes on which cells can grow while the membranes are stretched by means of vacuum.. This provides limited opportunity to control the imposed strain, and the Flexcell system was therefore modified to allow for a more detailed mechanical stimulation. The paper following this section describes the modifications done to the Flexcell system.

Myocytes growing on a membrane will scatter over the membrane, and the orientation of the muscle fibers formed by the myocytes will be random. This is not representative for real muscle tissue where contraction is made possible by alignment of fibers. Therefore, a method for aligning muscle cell cultures on the Flexcell membrane was developed, validated and tested in collaboration with Aalborg University's Stem Cell Laboratory (Pennisi et al., 2011)

9

Paper 4: Uniaxial cyclic strain drives assembly and differentiation of skeletal myocytes

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Uniaxial Cyclic Strain Drives Assembly and Differentiation of Skeletal Myocytes

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Ex vivo engineering of skeletal muscle represents an exciting new area of biotechnology. Although the ability of skeletal muscle cells to sense and respond to mechanical forces is well known, strategies based on the use of mechanical stimuli to optimize myogenesis *in vitro* remain limited. In this work, we describe a simple but powerful method based on uniaxial cyclic tensile strain (CTS) to induce assembly and differentiation of skeletal myocytes *in vitro.* Confluent mouse myoblastic precursors cultured on flexible-bottomed culture plates were subjected to either uniaxial or equibiaxial CTS. The uniaxial CTS protocol resulted in a highly aligned array of cross-striated fibers, with the major axis of most cells aligned perpendicularly to the axis of strain. In addition, a short period of myogenin activation and significant increase in the myotube/myoblast ratio and percentage of myosin-positive myotubes was found, indicating an enhanced cell differentiation. In contrast, cells under equibiaxial strain regimen had no clear orientation and displayed signs of membrane damage and impaired differentiation. These results, thus, demonstrate that the selection of a proper paradigm is a key element when discussing the relevance of mechanical stimulation for myogenesis *in vitro*. This study provides a rational framework to optimize engineering of functional skeletal muscle.

Introduction

Tissue engineered skeletal muscle is a highly desirable component for many biomedical applications, ranging from *in vitro* model systems for the study of muscle pathologies or drug screening[1–3] to *in vivo* applications including gene therapy and replacement of damaged tissue.[4–6] Current approaches to engineer skeletal muscle tissue *in vitro* are mostly based on the use of mouse C2C12[6–8] and rat L6 cell lines.[9–11] After being seeded on appropriate substrates, these myoblastic precursors are induced to fuse into myotubes that after a few days in culture mature into cells having some of the structural and functional characteristics of adult muscle fibers. It has been shown that both the phenotype and spatial organization of these myoblastic precursors can be optimized by changing the mechano-structural properties of the substrates on which cells are grown.[8,12–17] In addition to these static cues and given the role of strain in myogenesis *in vivo*,[18] it is believed that active mechanical stimulation is an important regulatory factor. However, the value of mechanical stimulation to induce the differentiation of mammalian skeletal muscle still remains unclear.

It has previously been reported that under a slowly rising uniaxial strain, embryonic chicken myoblasts were able to arrange in well-differentiated myotubes parallel to the direction of strain.[19] It is possible that this effect is species-specific, as mammalian myoblastic precursors failed to properly orient and differentiate when subjected to a similar stimulation protocol.[20,21] Subsequent studies subjected cells to cyclic tensile strain (CTS) by applying alternating phases of extension/relaxation, which appears to impart a remarkable effect on mammalian myoblastic precursors. The myoblasts undergoing uniaxial CTS respond by the induction of G-protein activation and increased protein synthesis.[22,23] In addition, they are able to rearrange themselves on the culture substrate,[24,25] which is consistent to the principle of actin fiber reorganization observed in many cell types.[26–30] Strikingly, however, neither the application of a uniform strain field in all directions, the equibiaxial CTS,[31–36] nor uniaxial CTS applied parallel to the long axis of cells[37] seem to support myogenesis. This experimental evidence has fueled the currently established concept of impairment of differentiation by mechanical stimulation of mammalian myoblastic precursors. Nevertheless, in the present study, we report on

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a novel paradigm of uniaxial CTS that has a significant positive effect on the assembly and differentiation of skeletal myocytes.

Materials and Methods

Cell culture

Mouse myogenic C2C12 cells were obtained from American Tissue Type Culture Collection (LGC Standards, Boras, Sweden). Growth medium consisted of 90% Dulbecco's modified Eagle medium (DMEM; Invitrogen), 10% fetal calf serum (Helena Bioscience), and 0.5% penicillin/streptomycin (Invitrogen). Differentiation medium consisted of DMEM medium supplemented with 2% heat-inactivated horse serum (Invitrogen) and 0.5% penicillin/streptomycin. Cell culture was performed in cell culture flasks (75 cm^2; BD Biosciences), and cells were passaged at 80%–90% confluency (every 3–4 days). Cells from passages 8 to 10 were removed from the culture flasks and seeded on 6-well flexible-bottom culture plates precoated with collagen-I (Bioflex, Flexcell, Dunn Labortechnik) at an approximate density of 5000 cells/cm^2. On confluence, cell differentiation was induced by substituting growth media by differentiation media (day 0). Differentiation media was replaced every 2 days.

Mechanical stimulation

Immediately after growth media was replaced by differentiation media, cells were subjected to mechanical stimulation for 48 h using a cell stretching device (Flexcell FX-5000; Flexcell). To subject cells to equibiaxial strain, standard circular pistons of 25 mm diameter were used, whereas for uniaxial strain, custom-made rectangular pistons were used. Rectangular pistons were designed using the finite element model described next and fabricated using a computer numerical-controlled milling machine. The mechanical stimulation protocol consisted of semi-sinusoidal tensile stretching pulses with a duration of 1 s. Peak amplitude was set to 15% in the stretching device. However, the actual values of maximum strain were smaller, as theoretically calculated and experimentally verified (Fig. 1b). Stimulation frequency was chosen as 0.5 Hz, which lies within the range adequate for cell alignment.[38] Rubber plugs (Flexstops; Flexcell) were used to block stretching in the wells used as controls.

Computational model and experimental validation

Finite element analysis allowed designing the rectangular loading post used for uniaxial strain stimulation. Strain distribution on the membrane for circular and rectangular pistons was calculated using the model after an experimental validation. The finite element model of the Flexcell system was built using the commercially available software Abaqus/CAE 6.9–2. The model was implemented as a circular membrane constrained on the edge. The membrane was modeled using geometrically nonlinear and linearly elastic, isotropic elements with a Young's modulus of 930 kPa and a nearly incompressible Poisson's ratio characteristic for the elastomer type of material from which the membrane is manufactured. Vacuum was applied to drape the membrane around the loading post, which was modeled using rigid shell elements. To validate the model, the level of applied vacuum was set to 66.49 kPa, which according to the manufacturer's specifications is the pressure difference delivered by the system to reach 20% deformation on a standard 25 mm loading post. To simulate the experimental conditions, the level of applied vacuum corresponded to a pressure difference of 50.31 kPa, which the Flexcell system applies to reach 15% strain on a standard 25 mm loading post. A matrix of 15×15 dots was plotted manually in one of the wells of a Flexcell culture plate (BioFlex BF-3001). The dots were separated at a horizontal/vertical distance of 2.0 ± 0.2 mm. The plate was mounted in the Flexcell system and placed under a binocular microscope (Leica Microsystems GmbH) equipped with a video camera (Sony Corporation). A strain regimen consisting of 20 steps ranging from 0% to 20% deformation was gradually applied to the plate, and images of the dotted membrane were taken at each step. A total of three series of images were taken for each piston type. Using the ImageJ v.1.43u image processing software (available at http://rsb.info.nih.gov/ij; National Institutes of Health), images were converted to gray scale, and a threshold was used to remove the background. The "analyze particles" algorithm was applied to the images to obtain the coordinates of the centroid of the pictures, which were exported to Microsoft Excel to calculate the translation of the dots under membrane deformation. The dots were configured into nine-node elements implemented using Abaqus/CAE 6.9-2, on which the registered node displacements were imposed. The finite elements subsequently interpolated the displacements and computed the strain field. After model validation, strain distributions calculated by the model were used to determine areas for cell image sampling, which were those areas where strain field was considered uniform, defined as that within a $\pm 1.5\%$ range.

Cell staining

After the culture period, cells were washed with phosphate-buffered saline solution (PBS) and fixed in 4% formaldehyde. Cell nuclei were stained using the Hoechst 33342 dye (Molecular Probes) for 30 min. Cells were permeabilized with 0.1% Triton X-100 in PBS and treated with 1% of bovine serum albumin to block nonspecific binding sites for antibodies. A Zenon Mouse IgG labeling kit (Alexa-fluor 647 Mouse IgG1; Molecular Probes) was used to stain a primary monoclonal anti-myosin antibody (Clone MY-32; Sigma-Aldrich A/S) according to the supplier's instructions. For staining fast skeletal myosin and myogenin, samples were incubated with a solution consisting of Zenon-labeled anti-myosin antibody (1:500) and Alexa-Fluor 488 anti-myogenin antibody (1:100; eBioscience). A second fixation was performed using 4% formaldehyde for 15 min. For staining of F-actin, cells were incubated with Bodipy 558/568 Phalloidin (2.5:100; Invitrogen). Finally, the samples were rinsed twice with PBS and kept in PBS at 4°C until the observation time.

Microscopy, image analysis, and counting

Phase-contrast brightfield images were obtained with an inverted microscope (CKX41; Olympus Danmark A/S) and a digital camera (PL-A782, Pixelink; Olympus Danmark A/S). Four-channel fluorescence images were obtained using an AxioCam digital camera (Carl Zeiss) attached to an Axio Observer.Z1 fluorescence microscope (Carl Zeiss). Image sampling was performed with the software AxioVision rel.

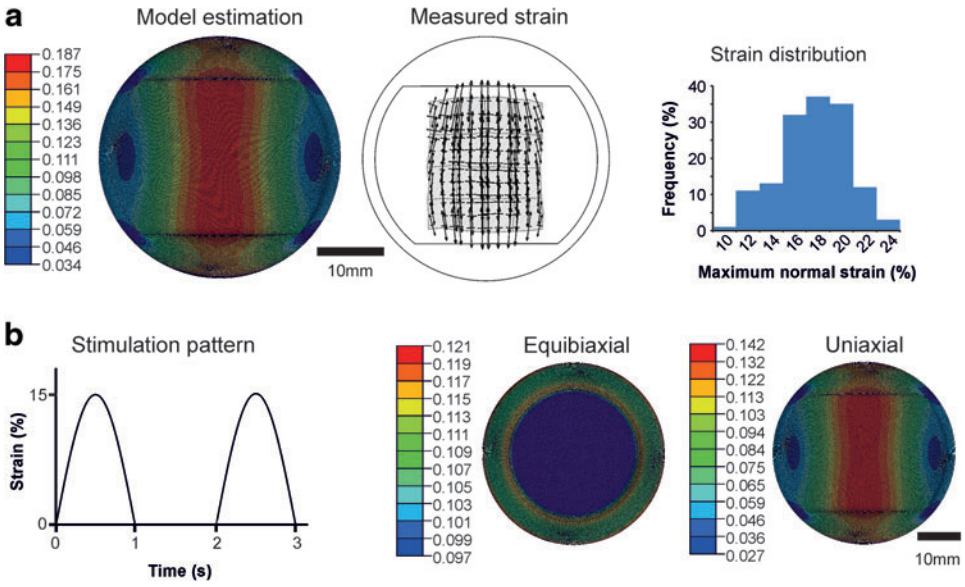

FIG. 1. Characteristics of the mechanical stimulation input. **(a)** Validation of the finite element model used to estimate strain distribution on the membrane for an input of 20%. The color map shows the estimation of the model, whereas the vector plot shows the results of the measurements. The histogram displays the magnitude and distribution of the maximum normal strain measured on the membrane. **(b)** Stimulation pattern used to induce cyclic tensile strain on the cells for 48 h. The graph shows one and a half cycle of the stimulation waveform, whereas the color maps display strain distribution on the membrane as calculated by the model for an input of 15% on equibiaxial and uniaxial regimens. Color scales in **a** and **b** represent maximum normal strain. Color images available online at www.liebertonline.com/tea

4.7 (Carl Zeiss). A total of 9 mosaic images consisting of 2×2 tiles taken at $20\times$ (Plan-Apochromat, Carl Zeiss) were acquired for each experimental condition. Resulting images, which covered an area of approximately 0.55 mm^2 with a resolution of 2674×1997 pixels, were used for cell counting, measurement of cell orientation, and qualitative analysis. For the analysis of actin and myosin cross-striations and membrane integrity, images were taken using a $63\times$ water immersion objective (C-Apochromat; Carl Zeiss). Cell counting was performed by two independent skilled observers to minimize the subjective bias, using the Cell Counter plug-in for the image processing software Image J. The parameters used for quantitative comparison of images were three: (1) the percentage of myogenin-positive nuclei, calculated from the number of myogenin-positive nuclei divided by the total number of nuclei, (2) the percentage of myosin-positive myotubes, calculated from the ratio of myotubes counted in the myosin channel divided by the number of myotubes counted in the actin channel, and (3) the myotube/myoblast ratio, calculated from the total number of myotubes divided by the number of myoblasts. The number of myoblasts was calculated as the difference between the total number of nuclei and the nuclei counted inside the myotubes. Cell alignment was quantified by using a custom written routine for AxioVision. Briefly, images were thresholded in the red channel (myosin staining), and for each cell, an object equivalent ellipse was calculated to estimate the orientation angle with regard to the horizontal axis.

Statistical analysis

For reproducibility verification, the experiments were performed with three biological replicates. Statistical analysis was performed using SPSS 17 (SPSS). Statistical differences in cell numbers were compared using one-way ANOVA. Unequal variances were assumed, as confirmed by Levene's homogeneity-of-variance test. *Post-hoc* comparisons among the groups were performed using Tamhane's T2 test.

Results

Calculation of the mechanical stimulation patterns

Figure 1a shows the result of the validation experiment performed for the finite element model of the Flexcell system. The strain contour shows the model estimation for the custom-made rectangular loading post when the system is set to produce 20% strain on a standard 25 mm circular post. It can be seen that under these conditions, the model predicts a rectangular area of approximately 2.6 cm^2 subjected to a unidirectional strain field ranging from 16.1% to 18.7%. Measurements of membrane deformation under these conditions are depicted by the vector plot and histogram on Figure 1a. It can be seen that vectors representing magnitude and direction of maximum normal strain are mostly parallel and range between 16% and 20% in magnitude. These measurements fit reasonably well with the strain field estimated by the model, validating a state of uniaxial strain in the considered area. Figure 1b displays the CTS stimulation pattern and strain contours calculated for an input of 15% strain on both circular and rectangular loading posts. It is shown that for the circular loading post, the principal strain has a uniform value of nearly 10% over a circular area of 5.6 cm^2, indicating a state of equibiaxial strain on that area. For the rectangular piston, the principal strain ranges from 12.2% to 14.2% in a rectangular area of 2.6 cm^2.

Following uniaxial CTS myotubes align in parallel

Immediately after exposure to the differentiation medium (day 0), the cells were subjected to a CTS regimen for 48 h. At the end of the mechanical stimulation period, cells were left in the incubator for 3 days to allow further differentiation. The representative phase contrast micrographs displaying the distribution and orientation of cells at days 0, 1, 2, and 5 are presented in Figure 2a. At day 0, myoblasts formed a dense monolayer of confluent mononucleated cells. From day 1 to 5, the cells in all groups underwent the differentiation

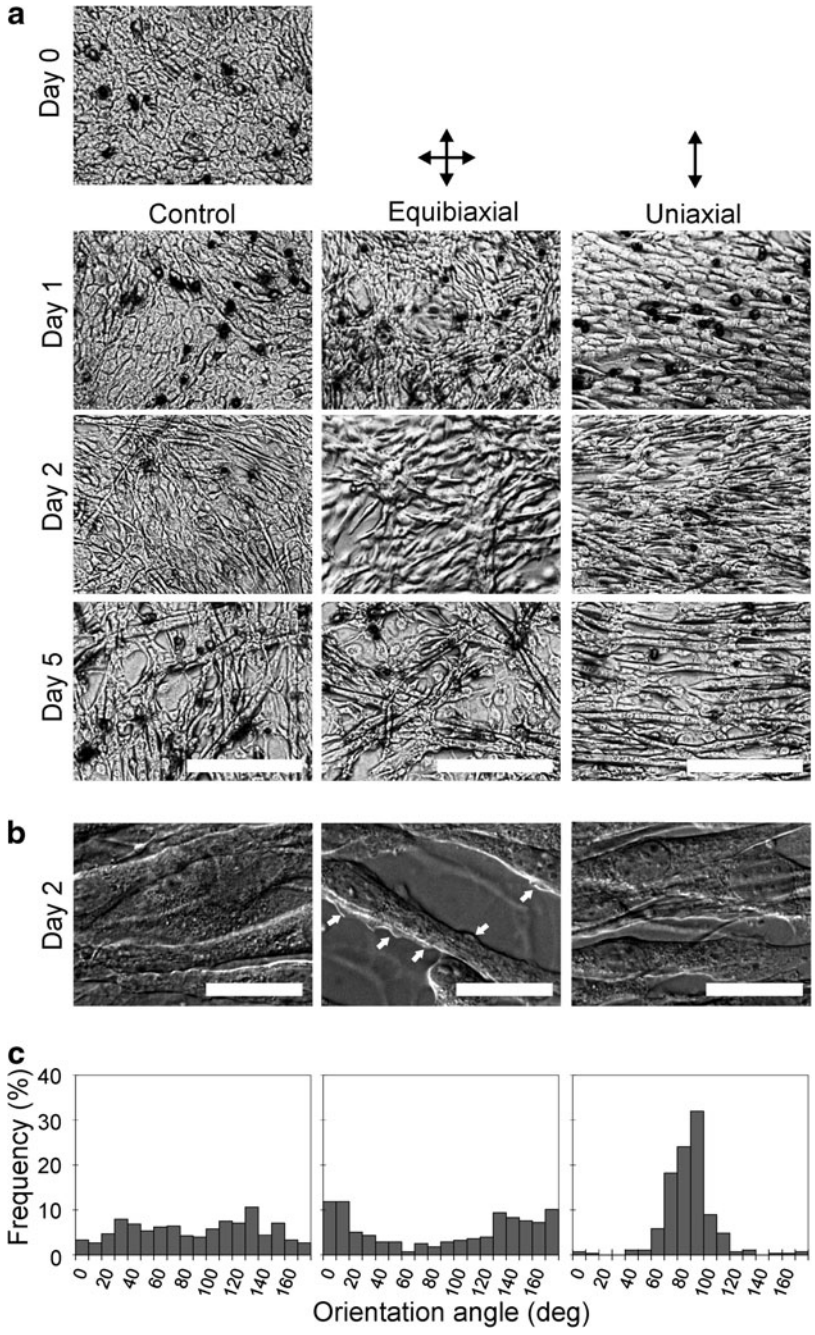

FIG. 2. Comparison of the effects of equibiaxial and uniaxial cyclic strain on cell orientation and morphology. **(a)** Phase-contast images showing cells after 1, 2, and 5 days under different experimental conditions. The direction of the strain is indicated by arrows. Scale bar denotes 200 μm **(b)** High-magnification phase-contrast images showing the typical plasmalemmal texture observed at day 2. Arrows indicate membrane blebs found in cells under equibiaxial strain. Scale bar denotes 50 μm. **(c)** Histograms displaying distributions of the myotube orientation angle at day 5 for one biological replicate. The histograms display the percentage of cells counted in a given angle interval throughout the nine images comprising the replicate.

process in a similar sequence: myoblast elongation, alignment, fusion into multinucleated myotubes, and myofibrilogenesis. Spontaneously contracting myotubes were found in all groups at day 5 (Supplementary Video S1, available online at www.liebertonline.com/tea). However, several differences between groups were found regarding number, size, and orientation of the cells. After 1 day under CTS, redistribution of the cells and morphological changes were already evident. Cells subjected to uniaxial CTS became elongated, and the major axis of most cells aligned perpendicularly to the axis of strain. In contrast, both cells in the unstrained controls and those under equibiaxial strain regimen had no clear orientation. At day 2, cells in all groups became more elongated, and some of them started to form myotubes, and the differences regarding cell orientation became more pronounced. Interestingly, the cells under equibiaxial strain tended toward arrays of radially oriented structures. In addition, these cells suffered membrane damage characterized by the presence of protruding blebs (Fig. 2b). At day 5, the process of myotube formation appeared to be concluded. The histomorphometric analysis of the differentiated cultures revealed that the average orientation angle was around 90°for cells under uniaxial CTS, whereas cells in the unstrained controls and those under equibiaxial strain regimen had no prevailing orientation (Fig. 2c).

CTS induced different levels of protein expression

The expression of two major sarcomeric proteins, the actin and myosin, as well as a marker of early myogenesis, the

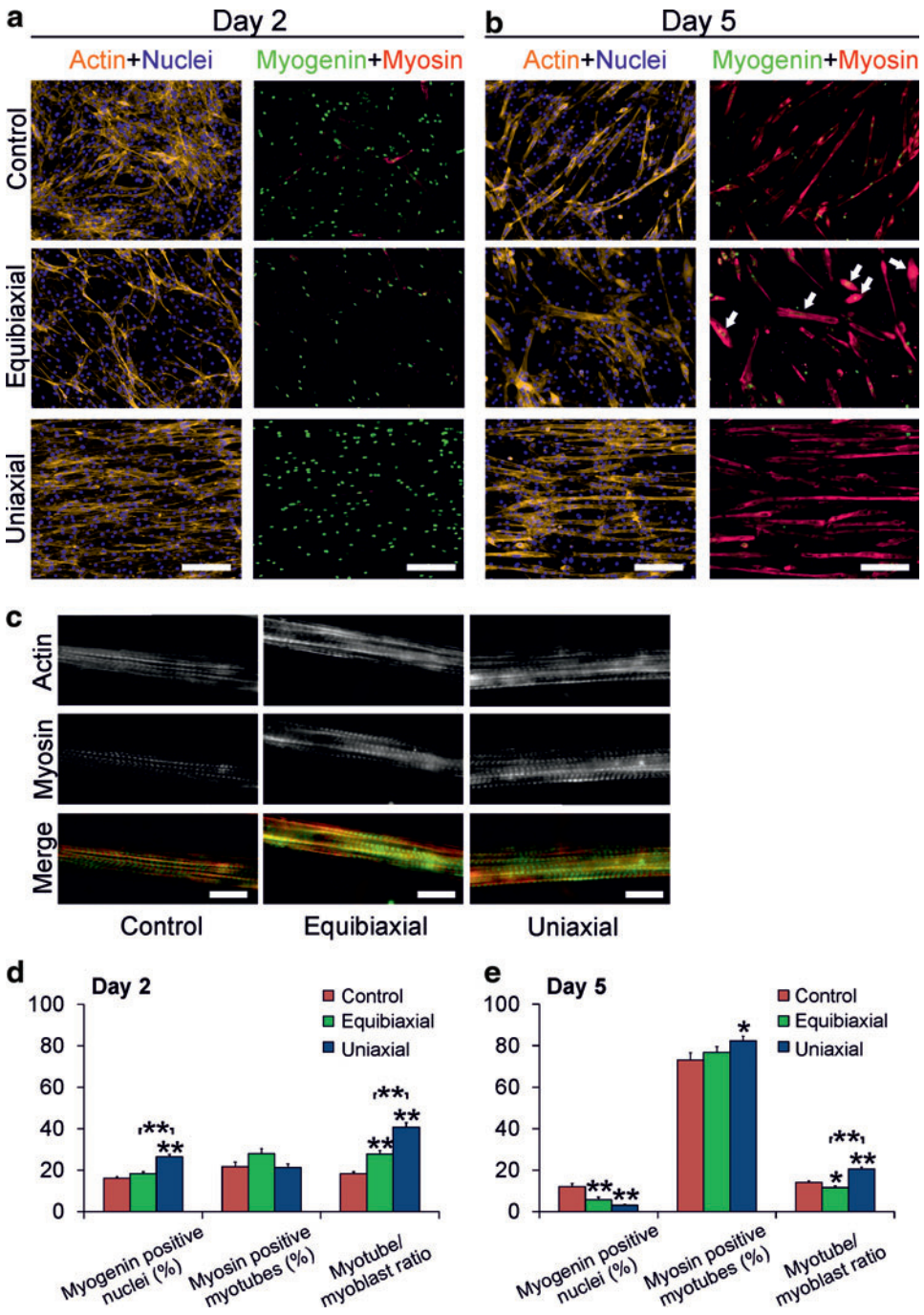

FIG. 3. Differentiation profiles following equibiaxial and uniaxial strain. **(a, b)** Representative fluorescence micrographs of myotubes at day 2 and 5. Cells were stained with Hoechst 33342 nuclear stain, Alexa-fluor 488 anti-myogenin antibody, Bodipy 558/568 phalloidin, and Alexa-fluor 647 labeled anti-myosin antibody. Arrows indicate small-sized myosin-positive myotubes frequently found on the samples under equibiaxial strain. Scale bar denotes 200 μm. **(c)** High-magnification fluorescence micrographs displaying actin and myosin striations on the different experimental conditions. The merged image was pseudocolored red for actin and green for myosin to improve the contrast. Scale bar denotes 20 μm. **(d, e)** Quantitative analysis of cell differentiation at day 2 and 5. Values are shown as mean ± standard error of the mean ($N = 3$). Statistically significant differences with regard to control and between mechanically stimulated cells are indicated by *($p < 0.001$) and **($p < 0.05$). Color images available online at www.liebertonline.com/tea

myogenin, was analyzed *in situ* (Fig. 3). On day 2, in cells subjected to uniaxial CTS, the actin stress fibers were aligned perpendicularly to the axis of strain, whereas in cells cultured under static mechanical conditions, the fibers had random orientation (Fig. 3a). Cells under equibiaxial CTS were clustered, and their actin stress fibers seemed to be radially oriented. Myogenin was expressed in a large number of cells undergoing differentiation in both the control and uniaxial strain groups, in contrast to myosin, which was only scarcely present throughout the cultures. On day 5, the progression of cell fusion was evidenced by the presence of multinucleated myotubes in all groups (Fig. 3b). Qualitative analysis of the actin and myosin expression patterns allowed comparison of the size of the myotubes (length and width). In the uniaxial CTS group, the size of the cells appeared to be similar to the control group. In contrast, shorter and thicker myotubes were frequently found in the equibiaxial group (as indicated by arrows on Fig. 3b). Apart from stress fibers, sarcomeric actin appeared in most of the myotubes in all groups. Myogenin positive nuclei were less evident, especially for cells under uniaxial CTS, and myosin staining was more profuse. The appearance of the latter was associated with the development of a fine sarcomeric architecture (Fig. 3c).

After uniaxial CTS, myotubes differentiate faster

Quantitative assessment confirmed the highest percentage of myogenin-positive nuclei and myotube-to-myoblast ratio

in the uniaxial CTS group at day 2 ($p < 0.001$) (Fig. 3d). The frequency of myosin-positive myotubes was comparable in all groups. On day 5, the tendency of myogenin activation was reversed, with uniaxially stretched cells featuring the smallest percentage of myogenin positive cells ($p < 0.001$) (Fig. 3e). The myotube-to-myoblast ratio remained highest for cells in the uniaxial CTS group ($p < 0.001$), but decreased below control levels in the equibiaxial CTS group ($p < 0.05$). The proportion of myosin-positive cells was highest in the uniaxial CTS group ($p < 0.05$), where it surpassed 80%. The increased early myogenin activation in the cells under the uniaxial CTS was clearly associated with accelerated and more complete maturation of the myotubes.

Discussion

In this work, a mechanical stimulation approach suitable for inducing alignment and differentiation of skeletal myocytes *in vitro* has been described. We have shown that five days after initiation of the differentitation protocol, cells consisted of a highly aligned array of cross-striated fibers, some of which displayed spontaneous contractions.

As shown in Fig. 1, cells under uniaxial CTS tend to align perpendicular to the direction of strain during the stimulation period (days 1 and 2) in contrast to nonstrained cells that did not show any particular orientation. These observations are consistent with the principle of actin stress fiber rearrangement in response to tensile forces. When cells are extended beyond a certain threshold, actin fibers adjust their length and orientation to an equilibrium level where extension is minimized. Cell reorientation via this mechanism has been demonstrated in various types of cultured cells.[26–30] For the C2C12 cell line in particular, Ahmed *et al.* have recently reported that uniaxial CTS (7% peak amplitude and 0.5 Hz frequency) caused myoblast reorientation to an angle of 70° with regard to the strain axis.[25] The process of cell reorientation appears to be proportional to the magnitude of the strain and may, thus, not always reach 90°.[24,39] Thus, the large displacement angle observed in this work (around 90°) could be explained by the higher level of strain experienced by the cells. Interestingly, we observed that cells under equibiaxial strain tended to form arrays of radially oriented cells protruding from the substrate plane. This response is consistent with the notion that stress fibers should only be formed in a direction that minimizes the longitudinal extension of the cells, which, in this case, is out of the plane of the membrane. Similar results, showing "tent-like" stress fiber structures, were reported for other types of cells under equibiaxial cyclic strain.[40] The presence of cells displaying signs of membrane damage (Fig. 2b) suggests that overstraining occurs even when the cells are arranged in these particular structures.

Besides producing cell alignment, uniaxial CTS caused faster cell differentiation. As shown in Figure 3, this was evidenced by a fast and short period of myogenin activation, a significant increase in the myotube/myoblast ratio throughout the observation period, and in the percentage of myosin-positive myotubes at day 5. In one of the few available reports applying CTS on myoblastic precursors, Clark *et al.* have shown that the mechanotransduction of physical forces is regulated by GTP binding proteins in C2C12 myoblasts.[23] Although nothing is mentioned in their study with regard to cell orientation and differentiation profiles, it is known that G-protein activation is linked to enhancement of myogenin expression and muscle differentiation,[41] which agrees with our findings. More recently, it has been shown that myoblasts that were grown on micropatterned surfaces and stimulated by CTS differentiated optimally when cells were oriented 45° with regard to the axis of strain. It was suggested that shear strain experienced by the cells was determinant in inducing sarcomeric development.[25] In contrast, cells in our experimental system were not constrained by geometrical cues on the substrate. However, we assume that some cells might have experienced large amounts of shear strain while migrating toward the perpendicular arrangement, as a consequence of the anisotropic strain applied to the membrane. Thus, it seems that non-equibiaxial strain is necessary to promote myogenesis, possibly due to the role of shear strain in the activation of mechanisms involved in myogenic differentiation.[23] In addition, the increase in the number of myosin-positive myotubes observed at day 5 might be explained by a stretch-mediated gene upregulation mechanism observed for cells under cyclic mechanical stimulation.[42,43]

In the equibiaxial CTS group, a reduced number of cells (Figs. 2 and 3), more myotubes than the control group at day 2, and a significant decrease of myogenin-positive nuclei at day 5 were found. Although some of these indicators seem to be associated with the differentiation process, they were accompanied by a significant decrease in the percentage of myotubes at day 5, which were shorter and thicker than those found in the other groups. The number of myosin-positive myotubes was not significantly different from the control group at days 2 and 5, which seems to disagree with previous reports showing that equibiaxial CTS is able to modulate the synthesis of myosin heavy chain isoforms.[42,43] This discrepancy might be due to the fact that this process is dependent on amplitude and frequency of the stimulation patterns. Overall, considering the membrane damage observed at day 2, these results indicate that equibiaxial CTS does not favor the differentiation process, which agrees with previous reports on the effect of equibiaxial CTS on differentiation of muscle precursor cells *in vitro*.[31–36] It was suggested that cyclic mechanical deformation could cause membrane damage leading to a sustained release of growth factors that favors cell proliferation and inhibits differentiation.[31] It is interesting to mention that these studies contributed to establish the concept that cyclic mechanical stimulation is not beneficial for strategies of *in vitro* muscle differentiation. Thus, although uniaxial CTS efficiently drives myogenesis, equibiaxial CTS does not favor the differentiation process, which is in agreement with the known fact that equibiaxial and uniaxial strain can provoke differential cellular responses in many cell types.[44,45] The results presented here highlight the importance of knowing the complete strain state of the cells, as the mechanical stimulation does not appear to be universally beneficial for *in vitro* muscle differentiation.

The parallel alignment of muscle fibers is one of the fundamental prerequisites for engineered functional skeletal muscle in order to mimic the *in vivo* tissue architecture and optimize force generation capacity. The most common approach for parallel arrangement of muscle cells for use in tissue engineering involves micropatterning parallel arrays

of topographical and/or biochemical features on the substrate surface.[8,12–16] These surface cues guide myoblast adhesion and orientation, which ultimately gives rise to parallel arrays of differentiated myotubes. However, fabrication of micropatterned substrates is a complex process that can be difficult to scale up to the large areas necessary for tissue engineering. In addition, cells that are not in direct contact with the substrate do not adopt the expected orientation.[12] The method outlined here seems to overcome these limitations. As shown in Figure 1a, uniform uniaxial strain has been applied over a relatively large membrane area (2.6 cm^2), which could, in principle, be increased by scaling up the mechanical stimulation system. Further, it is worth noting that unlike microtopography, which does not significantly influence the expression pattern of myogenic markers during myoblast differentiation,[46,47] the method presented here enhances the differentiation of myogenic precursors.

Although the principles presented here have been demonstrated using a two-dimensional culture system, we foresee its usefulness in tissue engineering applications aiming at obtaining three-dimensional muscle tissue. Given that appropriate means for cell detachment are procured, the two-dimensional myotube arrays could eventually be stacked to generate three-dimensional constructs, as it has been successfully applied in myoblast cell-sheet engineering for myocardial tissue reconstruction.[48] Another possibility could be to transfer the pre-aligned cell sheet onto a fibrin hydrogel and rely on the properties of self-organization of these aligned monolayers, as it was recently shown by Lam *et al.* for the fabrication of functional, three-dimensional, free-standing skeletal muscle.[49]

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Disclosure Statement

No competing financial interests exist.

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Paper 5: Elliptical posts allow for detailed control of Non-Equibiaxial straining of Cell Cultures

Elliptical Posts Allow for Detailed Control of Non-Equibiaxial Straining of Cell Cultures

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ABSTRACT
A modification of the Flexcell system that allows imposition of homogeneous, controlled non-equibiaxial strains to cell cultures is developed and experimentally validated. The Flexcell system by default applies equibiaxial strain to cell cultures, meaning no shear strain, while soft tissue cells *in vivo* are subjected to a range of mechanical deformations including shear strain caused by activities of daily living. Shear strains are suspected to play an important role in tissue necrosis. The Flexcell system was redesigned using a finite element model in order to obtain large areas of the membrane in a controlled, uniform non-equibiaxial strain state. The redesign was manufactured and the resulting strains were experimentally validated by means of image analysis methods. The result allows scientists and experimentalists to apply detailed control of the strain tensor applied to tissue samples in two dimensions.

Keywords: Flexcell, pressure ulcer, FEM,

INTRODUCTION

Cells from soft tissues, such as muscle, fat and skin, are continuously subjected to relatively large mechanical deformations caused by normal daily activities [8, 15]. Studies involving various types of cells subjected to mechanical strain have been essential to understanding the mechanisms underlying the normal and pathological cell responses to mechanical deformation [6, 13]. In certain pathological conditions, such as pressure ulcers or glaucoma, it has been hypothesized that cell death is caused simply by the deformation to which cells are subjected [4, 7, 9, 11, 12]. Since the deformation experienced by a cell during loading is a combination of several strain components, i.e. normal and shear strains assembled in the strain tensor, it was suggested that the combination of strains is an important factor in understanding how a cell response is related to the mechanical stimuli [3].

In order to study how mechanical stimuli affect cellular function, several devices have been developed to expose cells to uniaxial, equibiaxial, static or cyclic strain regimes [3]. One of the most frequently used systems for mechanostimulation of cells is the commercially available Flexcell tension apparatus (Flexcell International, Hillsborough, NC, USA.). In this system, a flexible membrane upon which the cell culture resides is stretched by draping it over a cylindrical loading post by means of internal vacuum. This subjects cells cultured on the membrane to uniform equibiaxial strain independent of orientation. However, most cells in natural environments are subjected to anisotropic strain, and this has motivated the search for alternative stimulation approaches that could more closely reproduce the *in vivo* conditions, such as those based on compressive indenters. Breuls et al. [2] described a flat glass indenter that was used to squeeze cells in culture, subjecting them simultaneously to compression, tension and shear strain. Gefen et al. [5] described an apparatus that indents a sphere into a lump of tissue, subjecting it to a compressive complex strain state comprising shear and tension strain in addition to compression. These studies provided valuable insight into the responses of cells to complex mechanical loads. However, in these studies the strain has not been considered as a tensor comprising normal as well as shear components, and therefore the parameters of the mechanical stimuli have not been fully quantified. It is therefore useful to develop a method to control the strain tensor in a systematic way to investigate the influence on the cell response.

Continuum mechanics provides an understanding of the complexities and coordinate system dependency of the strain tensor as well as a set of tools to simulate it over a continuum for instance in the Flexcell system [1, 14]. In this study, we shall use the finite element method (FEM) to redesign parts of the Flexcell tension 5000 system to allow for imposition of varying and controlled degrees of non-equibiaxial strain. The approach consists of a parametric FEM model of the Flexcell system built to analyze equipment modifications, which were subsequently experimentally validated.

The fundamental idea is to break the symmetry of the device by introduction of elliptical loading posts with different aspect ratios. The idea is inspired by the availability of analytical solutions in solid mechanics for uniformly loaded disks with elliptical holes, but the mechanics of the draped membrane is much more complicated and does not allow for an analytical solution. One of the complications is that the pressurized part of the membrane is what lies between the rim and the edge of the post, resulting in a design-dependent load on the system. Furthermore, the contact mechanics of the draping and the large deformations of the membrane complicate the model considerably.

The employed parametric finite element model allowed for identification of aspect ratios of oval loading posts that impart controlled magnitudes of shear strain, from zero through roughly half of the maximum normal strain over relatively large areas of the culture while keeping the maximum normal strain constant. Using the loading post aspect ratios reported in this work, scientists will be able to impose any ratio of shear to normal strains up to roughly 0.5.

METHODS

The study comprised an experimental and a modeling part (figure 1) focusing on the The Flexcell FX-5000 Tension System with 35 mm culture plates in six wells. The system is illustrated

in figure 2, where a single well is shown from the side in its standard configuration with a 25 mm cylindrical post.

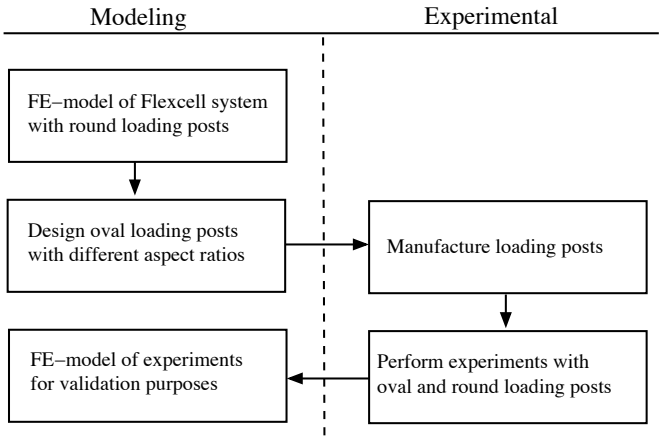

Figure 1 A schematic overview of the methodology used in the study.

A cylindrical loading post will cause the membrane to stretch equally in all directions, creating a two-dimensional hydrostatic or equibiaxial strain, which is characterized by absence of shear strain. Shear strain will be present in the membrane if a non-equibiaxial strain state is imposed. This was obtained in this study by reshaping the loading posts. Normal as well as shear strains are dependent on choice of coordinate system, but by means of continuum mechanics and Mohr's circle, the strain tensor can be rotated to its principal directions in which only principal strains, ε_1 and ε_2, where $\varepsilon_1 \geq \varepsilon_2$, exist and shear strains vanish. Rotating the coordinate system by another 45 degrees produces the directions in which the shear strains are maximal. These maximum shear strains can alternatively be expressed by the difference between the principal strains, $\varepsilon_1 - \varepsilon_2$,.

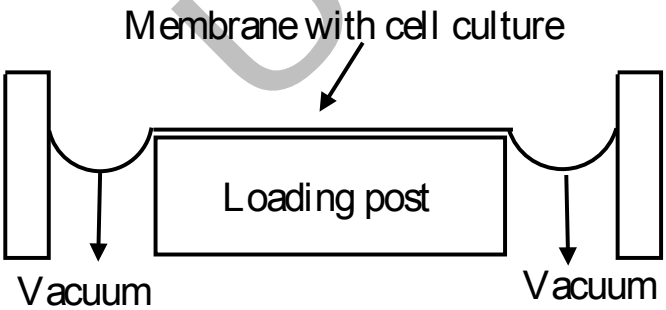

Figure 2 Illustration of Flexcell Tension system and 3-D FE-model used in the design process.

Computational model

Reshaping loading posts to obtain a desired strain state by experimental trial-and-error would require a very high number of experiments. Therefore, the loading posts were designed using the finite element method (FEM) as implemented in the commercially available software Abaqus/CAE 6.10-2 (Providence, RI, USA). The model was implemented as a circular membrane constrained on the edge. The membrane was modeled using geometrically non-linear, and linearly elastic, isotropic membrane elements with a Young's modulus of 930 kPa and a nearly incompressible Poisson's ratio, characteristic for the elastomer-type of material from which the membrane is manufactured. A vacuum was applied to drape the membrane around the loading post, which was modeled using rigid shell elements and the contact was considered frictionless. The vacuum applied corresponded to a pressure difference of 66.49 kPa, which the Flexcell system applies to reach 20% strain on a standard 25 mm loading post.

The objective of the design task was to obtain a series of geometries of the loading posts that produce a constant ε_1 over all the shape variations while the maximum shear strain, expressed as the principal strain difference $\varepsilon_1 - \varepsilon_2$, varies from zero to a maximum value i.e. from a cylindrical to an oval shaped loading post.

Experimental model validation

Four pistons were fabricated using a CNC-controlled milling machine. A matrix of 15 × 15 dots was plotted manually in one of the 35 mm wells of a Flexcell culture plate (BioFlex BF-3001). The dots were separated at a horizontal/vertical distance of 2 ± 0.5 mm (figure 3). Figure 3 depicts the dots on the end of the cylindrical 25 mm piston and the dots inside the square are those used to compute the strain field for the validation. The plate was mounted in the Flexcell system and placed under a binocular microscope (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a video camera (Sony Corporation, Tokyo, Japan). A straining regime consisting of 20 steps ranging from 0 to 20% deformation was gradually applied to the plate and images of the dotted well were taken at each step. A total of three series of images were taken for each piston type. Using the ImageJ v.1.43u image processing software (NIH, USA), images were converted to grey scale and a threshold was used to remove the background. The "Analyse particles" algorithm was applied to the images to obtain the coordinates of the centroid of the dots, which were exported to Microsoft Excel to calculate the

translation of the dots under membrane deformation. The dots were configured into 9-node elements implemented using Abaqus/CAE 6.9-2, on which the registered node displacements were imposed. The finite elements software subsequently interpolated the displacements and computed the strain field.

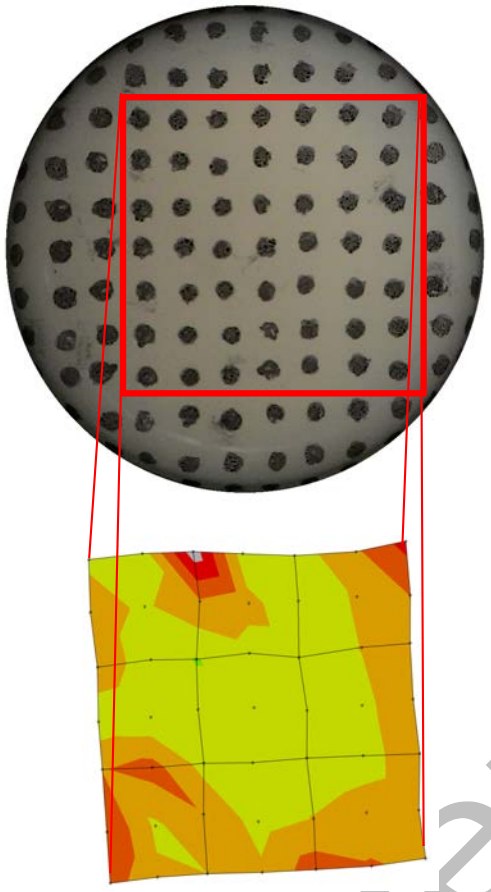

Figure 3 Membrane with dots used for validation.

RESULTS

Four new oval shaped pistons were designed and fabricated with an increasing axis ratio ranging from 1.06 to 1.23.

The effects of the pistons with different aspect ratios on the membrane strain are visualized in the columns of figure 4. The strains measured in the validation experiment are presented in table 1. The strains are comparable to the strains calculated by the FE-model of the different oval loading posts.

The experimental validation shows that the maximum normal strain for the differently shaped loading posts did not change significantly. The maximum shear strain on the other hand increased when going from cylindrical to oval shaped loading posts.

DISCUSSION

The objective of this study was to redesign the Flexcell tension system to apply shear strain in a controlled way to cell cultures. The loading posts of the system were redesigned, produced, and validated during this study. The strain applied to the membrane is a combination of tension, compression and shear and the combined strain is available for all positions on the membrane.

The newly designed posts provide a flexible but simple system to investigate the influence of shear strain on cell cultures, because their design allows for change of the shear strain only while the normal strain is kept constant. A state of "pure" shear strain immediately seems desirable for investigation of the effect of shear on the cells, but this is not physically possible, neither in the experiment nor *in-vivo*. The system limits the shear strain to approximately 50% of the normal strain but enables an all-things-equal scenario by keeping the normal strain constant. It is important to remember that strains are coordinate system dependent, so if the tissue under investigation has anisotropic properties, such as muscle or ligament, the alignment at the tissue on the membrane must be controlled. A recently published paper demonstrates how this can be obtained [10].

The experimental validation of the model provides a quantification of its validity, but the validation experiment itself is subject to inaccuracy as evident from the nonzero shear strain reported for the cylindrical post in Table 1. The principal source of experimental inaccuracy is the need to differentiate the registered point displacements to obtain strains. Despite this, the variation of maximum normal strain is rather small while the maximum shear strain is increasing with increasing axis ratios.

From the study it can be concluded that it is possible to change the Flexcell tension system to apply controlled shear strain to a cell culture, while keeping the maximum normal strain constant. This allows experimentalists to investigate the influence of shear strain on tissue in a systematic way, for example in the context of pressure ulcer research.

ACKNOWLEDGEMENTS

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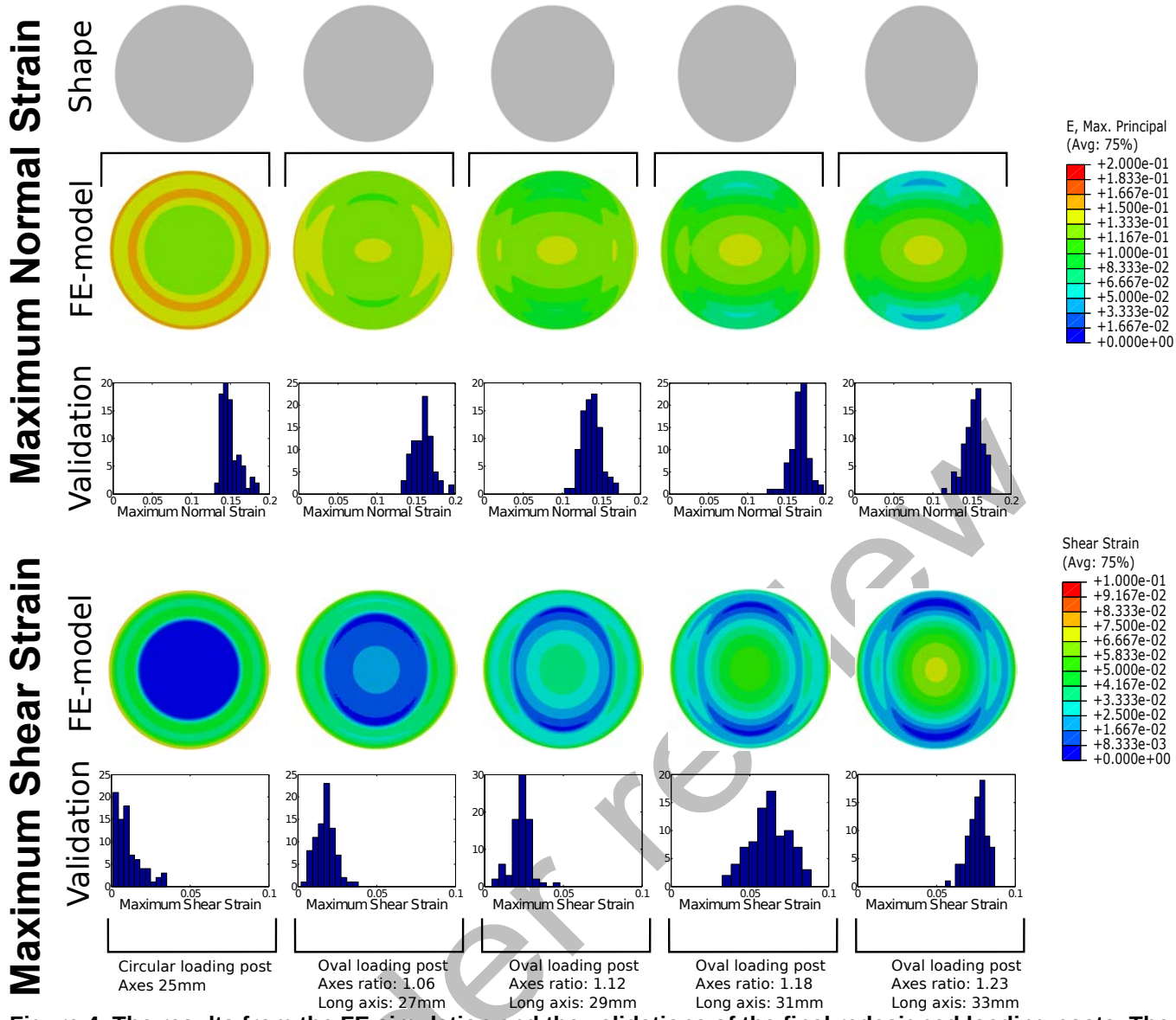

Figure 4 The results from the FE simulation and the validations of the final redesigned loading posts. The five different columns represent the cylindrical and the four oval shaped loading posts. The axis ratio is written under each column. The top row shows the FE simulation of maximum normal strain plots for the different shapes of loading posts. The second row in the figure shows the histogram of the experimental values of the maximum normal strain, indicating similar magnitudes over the different post shapes. The third row shows the FE simulation of maximum shear strain. Row four shows the histogram of the experimental values of the maximum shear strain level, indicating increasing shear strain levels with increasing axis ratios.

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11

Discussion and Summary of Papers

The problem in focus was to take a step towards better understanding of how the seated posture affects the tissue deformation and consequently tissue necrosis. The review paper included in the beginning concluded that three missing links should should be closed to gain a better understanding of the pressure ulcer problem.

The first point was to understand how the seated posture affects the reaction forces acting on the buttocks. A detailed seated musculo-skeletal model was developed in the AnyBody Modeling System and was validated with respect to measured reaction forces in chapter 5. An experimental setup was developed and data for three healthy subjects were collected and compared with the estimated forces calculated by the model. It would be beneficial to carry out similar experiments on people with disabilities. Wheelchair users often have postural problems, such as short hamstrings and scoliosis, in addition to the paralysis and these factors are not taken into account in the presented models. Doing so is experimentally feasible but more difficult than working with healthy test subjects due to the large variation of disability conditions of wheelchair users. The overall goal would be to make patient-specific models for wheelchair setup that would help optimizing the seated posture of individual wheelchair users in order to minimize risk of developing pressure ulcers.

The second point was to combine the musculoskeletal model with a FE-model of the buttocks, in order to investigate how the chair reaction forces affect the tissue strain, and especially how changes of chair reaction forces influence the tissue strain under the buttocks. This was investigated by using the seated FE-model developed by Wöelfel Beratende Ingeniure GmbH in combination with the seated AnyBody model. The investigated situation was the "Tilt-in-Space" wheelchair function that many wheelchairs, especially electrical wheelchairs, are equipped with. The study showed that the Tilt-in-Space function increased the shear force on the seat, while decreasing the normal force on the seat. The changed reaction forces on the seat induced a decrease in maximum shear strain and increased the maximum normal strain. Results demonstrated the complex relationship between input forces acting on a buttock and the strains experienced by the tissue. This work should be continued to investigate how changed posture in combination with different seat

cushion setup (changes stiffness or geometry properties) may prevent ulceration. This could become a strong methodology for designing optimized wheelchairs. Sitting in a wheelchair appears to be a combination of considerations of good posture adequate buttock support. The two factors are connected and inadequate focus on either one may lead to increased risk of developing a pressure ulcer.

The third point was to establish a cell death criterion for which the design and validation of the experimental setup was presented in chapter 8. This work should be followed by cell culture experiments where cultivated tissue should be subjected to different strain states. Methods for cultivating realistic striate muscle tissue with aligned fibers and for establishing controlled strain states were presented (Pennisi, C.P. et.al. 2010), but the experiment was not completed within the scope of this project due to problems with sustaining the attachment of the tissue on the membrane. Once this is accomplished, the link between posture and ulceration risk can finally be closed.

11.1 Concluding remarks

This PhD thesis and collection of papers have taken a step towards the understanding of how seated posture affects tissue deformation. Relating this to cell death has also been a focus area, but the final closing of the link between posture and ulceration risk has not yet been accomplished. Succeeding in doing so will increase the knowledge about pressure ulcer etiology significantly. No full understanding has yet been reached, but the present work closes significant gaps and confirm that the approach is a viable one. Future work should continue along the lines of the focal points discussed earlier, developing a strain-based cell death criterion that can be used as an output measure in FE-models of the human buttocks. The FE-models should also be developed further in terms of more detailed anatomy, the ability to deform to different postures and eventually patient-specific models. The musculoskeletal models require continued development in terms of subject-specificity and modeling of disabilities.

The generic models can be used by cushion designers to understand how tissue and cushion interact. The patient-specific models would be usable in clinical practice, if the tools become sufficiently handy and robust for this target group. This would allow for prescription of optimal and individually tailored wheelchairs based on an understanding of the biomechanics of the problem rather than the clinician's intuition.

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